

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
21 May 2004 (21.05.2004)

PCT

(10) International Publication Number
WO 2004/041852 A2

(51) International Patent Classification⁷: **C07K 14/16**

(21) International Application Number:
PCT/EP2003/012402

(22) International Filing Date:
3 November 2003 (03.11.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0225788.9 5 November 2002 (05.11.2002) GB

(71) Applicant (*for all designated States except US*): **GLAXO GROUP LIMITED** [GB/GB]; Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): **ERTL, Peter, Franz** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB).

(74) Agent: **PRIVETT, Kathryn, Louise**; GlaxoSmithKline (CN925.1), 980 Great West Road, Brentford, Middlesex TW8 9GS (GB).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: VACCINE

(57) Abstract: The invention relates to polynucleotides for DNA vaccination which polynucleotides encode an HIV envelope protein or fragment or immunogenic derivative, which is non-glycosylated when expressed in a mammalian target cell, operably linked to a heterologous promoter. Preferably the HIV envelope molecule, such as gp120 or gp140 or gp160, lacks a functional secretion signal. It may be fused to additional HIV proteins such as Nef, Gag, RT or Tat.

WO 2004/041852 A2

Vaccine

Field of the Invention

5 This invention relates to nucleic acid constructs, vectors comprising such constructs, methods of preparing the vectors and constructs and their use in prophylaxis or therapy, in particular therapeutic vaccines. The invention further relates to host cells comprising the constructs and vectors and to polypeptides encoded by the constructs as well as to the polypeptides per se. The invention further relates to pharmaceutical
10 formulations comprising the constructs and vectors and to the use of the constructs and vectors in medicine. The invention relates in particular to DNA vaccines that are useful in the prophylaxis and treatment of HIV infections, more particularly when administered by particle mediated delivery.

15 Background to the Invention

HIV-1 is the primary cause of the acquired immune deficiency syndrome (AIDS) which is regarded as one of the world's major health problems. Although extensive research throughout the world has been conducted to produce a vaccine, such efforts
20 thus far have not been successful.

The HIV envelope glycoprotein gp120 is the viral protein that is used for attachment to the host cell. This attachment is mediated by binding to two surface molecules of helper T cells and macrophages, known as CD4 and one of the two chemokine
25 receptors CCR-4 or CXCR-5. The gp120 protein is first expressed as a larger precursor molecule (gp160), which is then cleaved post-translationally to yield gp120 and gp41. The gp120 protein is retained on the surface of the virion by linkage to the gp41 molecule, which is inserted into the viral membrane.

30 The gp120 protein is the principal target of neutralizing antibodies, but unfortunately the most immunogenic regions of the proteins (V3 loop) are also the most variable parts of the protein. Therefore, the use of gp120 (or its precursor gp160) as a vaccine antigen to elicit neutralizing antibodies is thought to be of limited use for a broadly

protective vaccine. The gp120 protein does also contain epitopes that are recognized by cytotoxic T lymphocytes (CTL). These effector cells are able to eliminate virus-infected cells, and therefore constitute a second major antiviral immune mechanism. In contrast to the target regions of neutralizing antibodies some CTL epitopes appear to be relatively conserved among different HIV strains. For this reason gp120 and gp160 maybe useful antigenic components in vaccines that aim at eliciting cell-mediated immune responses (particularly CTL).

Non-envelope proteins of HIV-1 have been described and include for example internal structural proteins such as the products of the gag and pol genes and other non-structural proteins such as Rev, Nef, Vif and Tat (Green et al., New England J. Med, 324, 5, 308 et seq (1991) and Bryant et al. (Ed. Pizzo), *Pediatr. Infect. Dis. J.*, 11, 5, 390 et seq (1992).

HIV Tat and Nef proteins are early proteins, that is they are expressed early in infection and in the absence of structural protein.

The Nef protein is known to cause the removal of CD4, the HIV receptor, from the cell surface, but the biological importance of this function is debated. Additionally Nef interacts with the signal pathway of T cells and induces an active state, which in turn may promote more efficient gene expression. Some HIV isolates have mutations in this region, which cause them not to encode functional protein and are severely compromised in their replication and pathogenesis in vivo.

The Tat gene gives rise to a number of differentially spliced transcripts at different times during infection. The first exon encodes an 86 amino acid protein which dominates early in infection. The second exon encodes an additional 14 amino acids, and this partially spliced form of Tat is found late in infection. Both forms are fully functional transactivators, but the longer form also contains an RGD motif important for binding to $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins. Tat binds to a short-stem loop structure, known as the transactivation response element (TAR), that is located at the 5' terminus of HIV RNAs, and up-regulates transcription from the HIV LTR at least 1000-fold. Tat

has a role in promoting the elongation phase of HIV infection and stimulates the production of full-length viral transcripts. Tat can affect the expression of a number of cellular genes and can activate the expression of a number of cellular genes including TNF, IL-2 and IL-6, and regulates expression of p53 and Bcl-2. Tat is produced in
5 excess and is secreted from infected cells. This extra-cellular Tat can enter other cells and may prime cells for infection by HIV or accelerate the rate of HIV replication in newly infected cells.

In a conference presentation (C. David Pauza, Immunization with Tat toxoid
10 attenuates SHIV89.6PD infection in rhesus macaques, 12th Cent Gardes meeting, Marnes-La-Coquette, 26.10.1999), experiments were described in which rhesus macaques were immunised with Tat toxoid alone or in combination with an envelope glycoprotein gp160 vaccine combination (one dose recombinant vaccinia virus and one dose recombinant protein). The results observed showed that the presence of the
15 envelope glycoprotein gave no advantage over experiments performed with Tat alone.

The Gag gene is translated from the full-length RNA to yield a precursor polyprotein which is subsequently cleaved into 3 – 5 capsid proteins; the matrix protein, capsid protein and nucleic acid binding protein and protease. (1. Fundamental Virology,
20 Fields BN, Knipe DM and Howley M 1996 2. Fields Virology vol 2 1996).

The gag gene gives rise to the 55-kilodalton (kD) Gag precursor protein, also called p55, which is expressed from the unspliced viral mRNA. During translation, the N terminus of p55 is myristoylated, triggering its association with the cytoplasmic aspect
25 of cell membranes. The membrane-associated Gag polyprotein recruits two copies of the viral genomic RNA along with other viral and cellular proteins that triggers the budding of the viral particle from the surface of an infected cell. After budding, p55 is cleaved by the virally encoded protease (a product of the pol gene) during the process of viral maturation into four smaller proteins designated MA (matrix [p17]),
30 CA (capsid [p24]), NC (nucleocapsid [p9]), and p6.(4).

In addition to the 3 major Gag proteins, all Gag precursors contain several other regions, which are cleaved out and remain in the virion as peptides of various sizes.

These proteins have different roles e.g. the p2 protein has a proposed role in regulating activity of the protease and contributes to the correct timing of proteolytic processing.

5 The MA polypeptide is derived from the N-terminal, myristoylated end of p55. Most MA molecules remain attached to the inner surface of the virion lipid bilayer, stabilizing the particle. A subset of MA is recruited inside the deeper layers of the virion where it becomes part of the complex which escorts the viral DNA to the nucleus. These MA molecules facilitate the nuclear transport of the viral genome because a karyophilic signal on MA is recognized by the cellular nuclear import
10 machinery. This phenomenon allows HIV to infect non-dividing cells, an unusual property for a retrovirus.

The p24 (CA) protein forms the conical core of viral particles. Cyclophilin A has been demonstrated to interact with the p24 region of p55 leading to its incorporation
15 into HIV particles. The interaction between Gag and cyclophilin A is essential because the disruption of this interaction by cyclosporin A inhibits viral replication.

The NC region of Gag is responsible for specifically recognizing the so-called packaging signal of HIV. The packaging signal consists of four stem loop structures
20 located near the 5' end of the viral RNA, and is sufficient to mediate the incorporation of a heterologous RNA into HIV-1 virions. NC binds to the packaging signal through interactions mediated by two zinc-finger motifs. NC also facilitates reverse transcription.

25 The p6 polypeptide region mediates interactions between p55 Gag and the accessory protein Vpr, leading to the incorporation of Vpr into assembling virions. The p6 region also contains a so-called late domain which is required for the efficient release of budding virions from an infected cell.

30 The Pol gene encodes two proteins containing the two activities needed by the virus in early infection, the RT and the integrase protein needed for integration of viral DNA into cell DNA. The primary product of Pol is cleaved by the virion protease to yield the amino terminal RT peptide which contains activities necessary for DNA synthesis

(RNA and DNA directed DNA polymerase, ribonuclease H) and carboxy terminal integrase protein. HIV RT is a heterodimer of full-length RT (p66) and a cleavage product (p51) lacking the carboxy terminal Rnase integrase domain.

5 RT is one of the most highly conserved proteins encoded by the retroviral genome. Two major activities of RT are the DNA Pol and Ribonuclease H. The DNA Pol activity of RT uses RNA and DNA as templates interchangeably and like all DNA polymerases known is unable to initiate DNA synthesis de novo, but requires a pre existing molecule to serve as a primer (RNA).

10

The Rnase H activity inherent in all RT proteins plays the essential role early in replication of removing the RNA genome as DNA synthesis proceeds. It selectively degrades the RNA from all RNA - DNA hybrid molecules. Structurally the polymerase and ribo H occupy separate, non-overlapping domains with the Pol
15 covering the amino two thirds of the Pol.

The p66 catalytic subunit is folded into 5 distinct subdomains. The amino terminal 23 of these have the portion with RT activity. Carboxy terminal to these is the Rnase H Domain.

20

After infection of the host cell, the retroviral RNA genome is copied into linear ds DNA by the reverse transcriptase that is present in the infecting particle. The integrase (reviewed in Skalka AM '99 Adv in Virus Res 52 271-273) recognises the ends of the viral DNA, trims them and accompanies the viral DNA to a host
25 chromosomal site to catalyse integration. Many sites in the host DNA can be targets for integration. Although the integrase is sufficient to catalyse integration in vitro, it is not the only protein associated with the viral DNA in vivo - the large protein - viral DNA complex isolated from the infected cells has been denoted the pre integration complex. This facilitates the acquisition of the host cell genes by progeny viral
30 genomes.

The integrase is made up of 3 distinct domains, the N terminal domain, the catalytic core and the C terminal domain. The catalytic core domain contains all of the requirements for the chemistry of polynucleotidyl transfer.

5 DNA vaccines usually consist of a bacterial plasmid vector into which is inserted a strong promoter, the gene of interest which encodes an antigenic peptide and a polyadenylation/transcriptional termination sequence. The gene of interest may encode a full protein or simply an antigenic peptide sequence relating to the pathogen, tumour or other agent which it is intended to protect against. The plasmid can be
10 grown in bacteria, such as for example *E.coli* and then isolated and prepared in an appropriate medium, depending upon the intended route of administration, before being administered to the host. Following administration the plasmid is taken up by cells of the host, or delivered directly into the host cells, where the encoded peptide is produced. The plasmid vector will preferably be made without an origin of replication
15 functional in eukaryotic cells, in order to prevent plasmid replication in the mammalian host and integration within chromosomal DNA of the animal concerned.

There are a number of advantages of DNA vaccination relative to traditional vaccination techniques. First, it is predicted that because the proteins that are encoded
20 by the DNA sequence are synthesised in the host, the structure or conformation of the protein will be similar to the native protein associated with the disease state. It is also likely that DNA vaccination will offer protection against different strains of a virus, by generating a cytotoxic T lymphocyte response that recognises epitopes from conserved proteins. The technology also offers the possibility of combining diverse
25 immunogens into a single preparation to facilitate simultaneous immunisation in relation to a number of disease states.

Helpful background information in relation to DNA vaccination is provided in Donnelly et al "DNA vaccines" Ann. Rev Immunol. 1997 15: 617-648, the disclosure
30 of which is included herein in its entirety by way of reference.

Doe et al (1994) Eur J Immunol, 24: 2369-2376 investigated how variations in glycosylation affected the CD8+ CTL response to gp120 and found that gp120

produced in mammalian CHO cells had a reduced ability to prime CTL responses when compared with insect or yeast cell-derived envelope proteins unless N-linked oligosaccharides were removed prior to immunization.

5 It has now been discovered that there are benefits to be gained by employing a polynucleotide encoding a non-glycosylated HIV envelope protein in a vaccine for HIV. Surprisingly, a DNA vector expressing gp120 without a secretion signal and which is thus not glycosylated or secreted from the cell is a more effective stimulator of CTL responses than a DNA vector expressing gp120 with its native secretion
10 signal. Since the secretion signal is responsible for directing the gp120 to the intracellular site where glycosylation takes place, gp120 which lacks its native secretion signal is not glycosylated. Moreover, with the presence of a non-structural HIV protein such as Tat in a fusion protein with the non-glycosylated gp120, CTL responses to the gp120 are augmented. In contrast, Tat in a fusion protein with
15 normal gp120 prevents secretion but does not result in an augmented immune response. The non-glycosylated gp120 can also be successfully expressed in a fusion protein with other HIV antigens, both structural and non-structural.

Summary of the Invention

20

The present invention therefore provides novel constructs for use in nucleic acid or polypeptide vaccines for the prophylaxis and treatment of HIV infections and AIDS. In one aspect the invention provides a polynucleotide which comprises a sequence encoding an HIV envelope protein or fragment or immunogenic derivative thereof
25 which is non- or substantially non-glycosylated when expressed in a mammalian target cell, operably linked to a heterologous promoter.

30

Preferably the HIV envelope protein is gp120. Alternatively it may be other forms of the envelope protein such as gp160 or gp140.

HIV envelope proteins such as gp120 expressed in a mammalian cell will normally be glycosylated. According to the present invention the gp120 encoding sequence is adapted to reduce or prevent glycosylation in a mammalian target cell, particularly a

human target cell. Glycosylation may be reduced or prevented in a number of different ways, for example by removal of or mutation of the glycosylation sites or by removing the native secretion signal. Preferably in the polynucleotide construct according to the invention the gp120 or other form of HIV envelope protein lacks a functional secretion signal. The secretion signal may vary in length between HIV isolates, for example it is 30 amino acids long in the W61D isolate described herein, but may be more or less than that for different isolates. Generally the secretion signal is clearly delineated and will be removed in its entirety, although this is not necessarily the case. A sufficient amount of the signal will be removed to prevent its function of taking the envelope protein to the cellular machinery responsible for glycosylation. This can be easily tested for.

It will be understood that although the envelope protein does not have a functional secretion signal, there may still be a small amount of glycosylation taking place and this is not excluded from the invention. Thus the envelope protein according to the invention is substantially non-glycosylated in mammalian cells such that the majority of the envelope protein, for example greater than 50%, or preferably greater than 75%, or more preferably greater than 90%, or most preferably 95% or more of the envelope protein is not secreted from the cells.

The HIV envelope protein, particularly gp120 is preferably expressed as a fusion protein, preferably comprising at least one HIV protein selected from a non-structural protein such as Nef, Tat, and reverse transcriptase (RT) and a structural protein in particular a capsid protein such as Gag, or a fragment or immunogenic derivative of any of these.

In one embodiment the fusion protein is a gp120 and RT-containing fusion protein, optionally also comprising Gag and/or Nef.

In another embodiment the fusion protein is a gp120 and Gag-containing fusion protein optionally also comprising RT and/or Nef.

In a further embodiment the fusion protein is a gp120 and Nef-containing fusion protein optionally also comprising RT and/or Gag.

In the following preferred embodiments the fusion protein is a fusion of gp120, RT,
5 Nef and Gag or fragments or immunogenic derivatives thereof:

gp120-RT-Nef-Gag

RT-Nef-Gag-gp120

Another embodiment is a fusion comprising gp120 and Tat or fragments or
10 immunogenic derivatives thereof. In such an embodiment the polynucleotide according to the invention comprises a gp120 encoding sequence linked to a Tat encoding sequence to encode a gp120 and Tat-containing fusion protein.

In a particular embodiment the gp120 and Tat sequence is further linked to a Nef
15 encoding sequence to encode a gp120, Tat and Nef-containing fusion protein, most preferably a gp120-Nef-Tat fusion.

Additional HIV sequences may be included such as a Gag encoding sequence.

20 In another particular embodiment the fusion encoded by the polynucleotide according to the invention is a gp120-Gag-Nef-Tat fusion.

Preferably the Tat sequence for use in the invention is mutated so that it encodes a biologically inactive Tat which lacks transactivation activity but which maintains its
25 immunogenic epitopes. Tat transactivation activity can be measured for example by an HIV LTR reporter system such as a CAT assay system in which the chloramphenicol-acetyl transferase reporter gene is under the control of the long terminal repeat of HIV-1. One specific CAT assay which is suitable uses the HL3T1 cell line which is described in Felber and Pavlakis (1988) Science 239: 184-187 and
30 also used in Mischiati et al (2001) Antisense Nucleic Acid Drug Dev Aug 11 (4): 209-17. HL3T1 is a HeLa cell line which contains stably integrated silent copies of HIV-1 LTR promoter linked to the CAT gene. These cells are transfected with DNA vectors

containing the Tat gene, modified or not. CAT is produced upon presence of an active Tat and measured by ELISA.

One preferred mutated Tat sequence (originating from BH10 molecular clone) bears
5 mutations in the active site region (Lys41→Ala) and in the RGD motif (Arg78→Lys and Asp80→Glu) (Virology 235: 48-64, 1997).

Optionally the Nef sequence for use in the invention is truncated to remove the sequence encoding the N terminal region i.e. removal of 30-85, preferably 60-85,
10 preferably the N terminal 65 amino acids (the latter truncation is referred to herein as trNef). Advantageously the Nef may be modified to remove one or more myristylation sites. For example the Gly 2 myristylation site may be removed by deletion or substitution. Alternatively or additionally the Nef may be modified to alter the dileucine motif of Leu 174 and Leu 175 by deletion or substitution of one or both
15 leucines. The importance of the dileucine motif in CD4 downregulation is described e.g. in Bresnahan P.A. et al (1998) Current Biology, 8(22): 1235-8.

Preferably the Gag for use in the invention does not encode the Gag p6 polypeptide. Preferred Gag sequences for use in the invention comprise P17 and/or 24.

20

The RT polynucleotide for use in the invention preferably encodes a mutation to substantially inactivate any reverse transcription activity. A preferred inactive mutant involves the substitution of W tryptophan 229 for K lysine. See WO 03/025003.

25 Preferably one or more of the HIV sequences included in the polynucleotide according to the invention encoding e.g. gp120, Nef, Tat, Gag or RT is or are codon optimised for mammalian cells, most preferably such that it/they resemble a highly expressed human gene in their codon use.

30 The fusion may contain further HIV sequences. It will be understood that for all of the HIV sequences included in the invention, these do not necessarily represent sequences encoding the full length or native proteins. Immunogenic derivatives such

as truncated or otherwise altered e.g. mutated proteins are also contemplated, as are fragments which encode at least one HIV epitope, preferably a CTL epitope, typically a peptide of at least 8 amino acids. Immunogenic derivatives of the fragments are also contemplated. Immunogenic derivatives cross react with the native antigen or
 5 fragment thereof. Polynucleotides which encode a fragment of at least 8, for example 8-10 amino acids or up to 20, 50, 60, 70, 100, 150 or 200 amino acids in length are considered to fall within the scope of the invention as long as the encoded oligo or polypeptide demonstrates HIV antigenicity. The HIV polypeptide molecules encoded by the polynucleotide sequences according to the invention preferably represent a
 10 fragment of at least 50% of the length of the native protein, which fragment may contain mutations but which retains at least one HIV epitope and demonstrates HIV antigenicity. Similarly, immunogenic derivatives according to the invention must demonstrate HIV antigenicity. Preferred immunogenic derivatives provide some potential advantage over the native protein such as reduction or removal of a function
 15 of the native protein which is undesirable in a vaccine antigen such as an enzyme activity (RT), transactivating activity (Tat), or CD4 downregulation (Nef). The polynucleotide sequences are preferably codon optimised for mammalian cells, in line with preferred aspects of the invention.

20 Preferred polynucleotide sequences are selected from the group:

1. gp120 codon optimised, minus secretion signal
2. gp120 codon optimised, minus secretion signal – tr Nef
3. gp120 codon optimised, minus secretion signal – tr Nef – mTat
- 25 4. gp120 codon optimised, minus secretion signal – Nef - mTat
5. gp120 codon optimised, minus secretion signal – p17/24 Gag – tr Nef
6. gp120 codon optimised, minus secretion signal – p17/24 Gag – tr Nef - mTat
7. gp120 codon optimised, minus secretion signal - p17/24 Gag - Nef-mTat
8. gp120 codon optimised, minus secretion signal - p17/24 Gag - mNef-mTat
- 30 9. gp120 codon optimised, minus secretion signal - p17/24 Gag - L1Nef-mTat
10. gp120 codon optimised, minus secretion signal - p17/24 Gag - L2Nef-mTat
11. gp120 codon optimised, minus secretion signal - p17/24 Gag - LLNef-mTat
12. gp120 codon optimised, minus secretion signal - p17/24 Gag - mLLNef-mTat

13. gp120 codon optimised, minus secretion signal - p17/24 Gag - mL1Nef-mTat
14. gp120 codon optimised, minus secretion signal - p17/24 Gag - mL2Nef-mTat
15. gp120 codon optimised, minus secretion signal - mRT- trNef - p17/24 Gag
16. mRT – trNef – p17/24 Gag – gp120 codon optimised, minus secretion signal

5

mNef = deletion of G2 to give non-myristoylated Nef

L1 Nef = L174A mutation in Nef

L2Nef = L175A mutation in Nef

LLNef = L174A and L175A mutations in Nef

- 10 TrNef = Nef devoid of nucleotides encoding terminal amino acids 1-65

mRT = Reverse Transcriptase mutated to remove biological activity (W229K). RT is codon optimised

Gag = Gag codon optimised.

- 15 The invention preferably relates to HIV-1. It is preferred that the constructs described herein are derived from an HIV clade B or clade C, particularly clade B.

Preferably the promoter is the promoter from HCMV IE gene, more particularly wherein the 5' untranslated region of the HCMV IE gene comprising exon 1 is

- 20 included as described in WO 02/36792.

In another aspect the invention provides a vector comprising the polynucleotide sequences described herein. The polynucleotide sequence is preferably DNA and is preferably contained within a vector which is a double stranded DNA plasmid .

- 25 Alternative vectors are described hereinbelow and include in particular adenovirus vectors such as chimp derived adenovirus vectors Pan 9 or Pan 5, 6 and 7, preferably where these are replication defective such that they cannot replicate in the target cells.

- 30 Another aspect of the invention relates to a set of polynucleotides encoding an HIV envelope protein or fragment or immunogenic derivative thereof, particularly gp120, and separately one or more other HIV proteins such as RT, Gag, Nef, Tat or fragments or immunogenic derivatives thereof.

Thus the invention provides a set of polynucleotides comprising a polynucleotide as described herein for gp120 or another form of the HIV envelope protein, and at least one further polynucleotide encoding at least one of HIV Nef, Gag, RT or Tat or fragment or immunogenic derivative thereof, optionally linked to an additional
 5 promoter.

Preferably the set of polynucleotides is contained on a single vector, in particular a DNA plasmid, although other vectors such as adenovirus as described herein are also contemplated. Preferably the polynucleotides are under the control of two or more
 10 separate promoters. Alternatively the two polynucleotides may be under the control of a single promoter, and optionally translation of the second polynucleotide may be enhanced by inclusion of an internal ribosome entry site between the polynucleotides.

Preferably the set of polynucleotides comprises a gp120 polynucleotide as described
 15 herein and a further polynucleotide encoding a fusion of RT-Nef-Gag or of fragments or immunogenic derivatives thereof.

Preferred sets of polynucleotides are selected from the group:

- 20 1. gp120 codon optimised, minus secretion signal + tr Nef-mTat
2. gp120 codon optimised, minus secretion signal + P17/24 Gag - tr Nef
3. gp120 codon optimised, minus secretion signal + P17/24 Gag - Nef - mTat
4. mRT - tr Nef - P17/24 Gag + gp120 codon optimised, minus secretion signal
5. gp120 codon optimised, minus secretion signal + mRT - tr Nef - P17/24 Gag

25

Where these preferred sets of two polynucleotides are contained on a single vector under the control of two separate promoters, this may be referred to as a dual promoter vector.

30 The preferred promoter for one or both of the protein or fusion proteins in a set of polynucleotides according to the invention is the promoter from HCMV IE gene, more particularly wherein the 5' untranslated leader sequence comprises exon 1 as described in WO 02/36792, as described herein.

In yet another aspect the invention provides polypeptides encoded by the polynucleotides and vectors described herein.

- 5 In one embodiment the invention provides a fusion protein comprising substantially non-glycosylated HIV envelope protein or a fragment or immunogenic derivative thereof and at least one additional HIV protein or fragment or immunogenic derivative thereof said additional HIV protein selected from Nef, Gag, RT and Tat.
- 10 In another embodiment the invention provides a composition comprising a substantially non-glycosylated HIV envelope protein or a fragment or immunogenic derivative thereof and at least one additional HIV protein or fragment or immunogenic derivative thereof said at least one additional HIV protein selected from Nef, Gag, RT and Tat. Preferably the at least one additional HIV protein is a fusion protein of two
- 15 or more of Nef, Gag, RT and Tat or fragments or immunogenic derivatives thereof.

In yet another embodiment the invention provides a substantially non-glycosylated HIV envelope protein or a fragment or immunogenic derivative thereof, expressed from a polynucleotide which is codon optimised for mammalian cells.

20

- In a further aspect the invention provides pharmaceutical compositions comprising the nucleotide sequences and vectors and polypeptides described herein, together with a pharmaceutically acceptable excipient, diluent, carrier or adjuvant. In a preferred embodiment the polynucleotide, preferably in the form of a DNA vector and
- 25 preferably comprising at least one codon optimised HIV sequence, is present in a composition comprising a plurality of particles, preferably beads such as gold beads, onto which the DNA is coated.

- Delivery of polynucleotides according to the invention is preferably carried out by
- 30 particle mediated delivery, particularly via a bombardment approach.

It is envisaged that the vectors according to the invention may be utilised with immunostimulatory agents, preferably but not necessarily administered at the same

time as the vectors and preferably formulated together in the compositions according to the invention.

Immunostimulatory agents for use in the invention include, but this list is by no means
5 exhaustive and does not preclude other agents: synthetic imidazoquinolines such as
imiquimod [S-26308, R-837], (Harrison, et al. 'Reduction of recurrent HSV disease
using imiquimod alone or combined with a glycoprotein vaccine', Vaccine 19: 1820-
1826, (2001)); and resiquimod [S-28463, R-848] (Vasilakos, et al. 'Adjuvant activities
of immune response modifier R-848: Comparison with CpG ODN', Cellular
10 immunology 204: 64-74 (2000).), Schiff bases of carbonyls and amines that are
constitutively expressed on antigen presenting cell and T-cell surfaces, such as
tucarecol (Rhodes, J. et al. 'Therapeutic potentiation of the immune system by
costimulatory Schiff-base-forming drugs', Nature 377: 71-75 (1995)), cytokine,
chemokine and co-stimulatory molecules as either protein or peptide or DNA, this
15 would include pro-inflammatory cytokines such as GM-CSF, IL-1 alpha, IL-1 beta,
TGF- alpha and TGF - beta, Th1 inducers such as interferon gamma, IL-2, IL-12, IL-
15 and IL-18, Th2 inducers such as IL-4, IL-5, IL-6, IL-10 and IL-13 and other
chemokine and co-stimulatory genes such as MCP-1, MIP-1 alpha, MIP-1 beta,
RANTES, TCA-3, CD80, CD86 and CD40L, other immunostimulatory targeting
20 ligands such as CTLA-4 and L-selectin, apoptosis stimulating proteins and peptides
such as Fas, (49), synthetic lipid based adjuvants, such as vaxfectin, (Reyes et al.,
'Vaxfectin enhances antigen specific antibody titres and maintains Th1 type immune
responses to plasmid DNA immunization', Vaccine 19: 3778-3786) squalene, alpha-
tocopherol, polysorbate 80, DOPC and cholesterol, endotoxin, [LPS], Beutler, B.,
25 'Endotoxin, 'Toll-like receptor 4, and the afferent limb of innate immunity', Current
Opinion in Microbiology 3: 23-30 (2000)) ; CpG oligo- and di-nucleotides, Sato, Y. et
al., 'Immunostimulatory DNA sequences necessary for effective intradermal gene
immunization', Science 273 (5273): 352-354 (1996). Hemmi, H. et al., 'A Toll-like
receptor recognizes bacterial DNA', Nature 408: 740-745, (2000) and other potential
30 ligands that trigger Toll receptors to produce appropriate Th1-inducing cytokines,
such as synthetic Mycobacterial lipoproteins, Mycobacterial protein p19,
peptidoglycan, teichoic acid and lipid A.

A preferred immunostimulatory agent for use with the invention is GM-CSF. This may be employed in the form of a polynucleotide expressing GM-CSF which is co-administered with the DNA vaccine of the invention. A DNA plasmid encoding GM-CSF may be present in a pharmaceutical composition comprising the
5 polynucleotide(s) according to the invention.

Certain preferred adjuvants for eliciting a predominantly Th1-type response include, for example, a Lipid A derivative such as monophosphoryl lipid A, or preferably 3-de-O-acylated monophosphoryl lipid A. MPL[®] adjuvants are available from Corixa
10 Corporation (Seattle, WA; *see*, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory
15 DNA sequences are also described, for example, by Sato et al., *Science* 273:352, 1996. Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins.

20 According to a further aspect of the invention, a host cell comprising a polynucleotide sequence according to the invention, or an expression vector according to the invention, is provided. The host cell may be for example bacterial e.g. *E. coli*, mammalian e.g. human, or may be an insect cell. Mammalian cells comprising a vector according to the present invention may be cultured cells transfected *in vitro* or
25 may be cells transfected *in vivo* by administration of the vector to the mammal.

By codon optimisation is meant that the DNA sequence is optimised to resemble the codon usage of genes in mammalian cells. In particular, the codon usage in the sequence is optimised to resemble that of highly expressed human genes.

30

The DNA code has 4 letters (A, T, C and G) and uses these to spell three letter “codons” which represent the amino acids the proteins encoded in an organism’s genes. The linear sequence of codons along the DNA molecule is translated into the

linear sequence of amino acids in the protein(s) encoded by those genes. The code is highly degenerate, with 61 codons coding for the 20 natural amino acids and 3 codons representing "stop" signals. Thus, most amino acids are coded for by more than one codon - in fact several are coded for by four or more different codons.

5

Where more than one codon is available to code for a given amino acid, it has been observed that the codon usage patterns of organisms are highly non-random. Different species show a different bias in their codon selection and, furthermore, utilisation of codons may be markedly different in a single species between genes which are expressed at high and low levels. This bias is different in viruses, plants, bacteria and mammalian cells, and some species show a stronger bias away from a random codon selection than others. For example, humans and other mammals are less strongly biased than certain bacteria or viruses. For these reasons, there is a significant probability that a mammalian gene expressed in *E. coli* or a foreign or recombinant gene expressed in mammalian cells will have an inappropriate distribution of codons for efficient expression. It is believed that the presence in a heterologous DNA sequence of clusters of codons or an abundance of codons which are rarely observed in the host in which expression is to occur, is predictive of low heterologous expression levels in that host.

20

In an embodiment of the present invention there is provided a gp120 polynucleotide sequence which encodes a substantially non-glycosylated gp120 amino acid sequence, wherein the codon usage pattern of the polynucleotide sequence resembles that of highly expressed mammalian genes. Preferably the polynucleotide sequence is a DNA sequence. Desirably the codon usage pattern of the polynucleotide sequence is typical of highly expressed human genes.

25

In the polynucleotides of the present invention, the codon usage pattern is altered from that typical of human immunodeficiency viruses to more closely represent the codon bias of the target organism, e.g. a mammal, especially a human. The "codon usage coefficient" is a measure of how closely the codon pattern of a given polynucleotide sequence resembles that of a target species. Codon frequencies can be derived from literature sources for the highly expressed genes of many species (see e.g. Nakamura

30

et.al. Nucleic Acids Research 1996, 24:214-215). The codon frequencies for each of the 61 codons (expressed as the number of occurrences occurrence per 1000 codons of the selected class of genes) are normalised for each of the twenty natural amino acids, so that the value for the most frequently used codon for each amino acid is set to 1 and
5 the frequencies for the less common codons are scaled to lie between zero and 1. Thus each of the 61 codons is assigned a value of 1 or lower for the highly expressed genes of the target species. In order to calculate a codon usage coefficient for a specific polynucleotide, relative to the highly expressed genes of that species, the scaled value for each codon of the specific polynucleotide are noted and the geometric mean of all
10 these values is taken (by dividing the sum of the natural logs of these values by the total number of codons and take the anti-log). The coefficient will have a value between zero and 1 and the higher the coefficient the more codons in the polynucleotide are frequently used codons. If a polynucleotide sequence has a codon usage coefficient of 1, all of the codons are "most frequent" codons for highly
15 expressed genes of the target species.

According to the present invention, the codon usage pattern of the polynucleotide will preferably exclude rare codons. Rare codons can be defined as codons representing <20% or more preferably representing <10% of the codons used for a particular amino
20 acid in highly expressed genes of the target organism. Alternatively rare codons may be defined as codons with a relative synonymous codon usage (RSCU) value of <0.3 or more preferably <0.2 in highly expressed genes of the target organism. An RSCU value is the observed number of codons divided by the number expected if all codons for that amino acid were used equally frequently. An appropriate definition of a rare
25 codon would be apparent to a person skilled in the art.

A polynucleotide of the present invention will generally have a codon usage coefficient for highly expressed human genes of greater than 0.3, preferably greater than 0.4, most preferably greater than 0.5. Preferably also the codon usage coefficient
30 will be less than 1.0, preferably less than 0.9 and more preferably less than 0.8. Thus a codon usage coefficient between 0.5 and 0.9 or between 0.5 and 0.8 is most preferred. Codon usage tables for human can also be found in Genbank.

In comparison, a highly expressed beta actin gene has a codon usage coefficient of 0.747.

The codon usage table for a homo sapiens is set out below:

5

Codon usage for human (highly expressed) genes 1/24/91 (human_high.cod)

| | AmAcid | Codon | Number | /1000 | Fraction | .. |
|----|--------|-------|---------|-------|----------|----|
| 10 | Gly | GGG | 905.00 | 18.76 | 0.24 | |
| | Gly | GGA | 525.00 | 10.88 | 0.14 | |
| | Gly | GGT | 441.00 | 9.14 | 0.12 | |
| | Gly | GGC | 1867.00 | 38.70 | 0.50 | |
| 15 | Glu | GAG | 2420.00 | 50.16 | 0.75 | |
| | Glu | GAA | 792.00 | 16.42 | 0.25 | |
| | Asp | GAT | 592.00 | 12.27 | 0.25 | |
| | Asp | GAC | 1821.00 | 37.75 | 0.75 | |
| 20 | Val | GTG | 1866.00 | 38.68 | 0.64 | |
| | Val | GTA | 134.00 | 2.78 | 0.05 | |
| | Val | GTT | 198.00 | 4.10 | 0.07 | |
| | Val | GTC | 728.00 | 15.09 | 0.25 | |
| 25 | Ala | GCG | 652.00 | 13.51 | 0.17 | |
| | Ala | GCA | 488.00 | 10.12 | 0.13 | |
| | Ala | GCT | 654.00 | 13.56 | 0.17 | |
| | Ala | GCC | 2057.00 | 42.64 | 0.53 | |
| 30 | Arg | AGG | 512.00 | 10.61 | 0.18 | |
| | Arg | AGA | 298.00 | 6.18 | 0.10 | |
| | Ser | AGT | 354.00 | 7.34 | 0.10 | |
| | Ser | AGC | 1171.00 | 24.27 | 0.34 | |
| 35 | Lys | AAG | 2117.00 | 43.88 | 0.82 | |
| | Lys | AAA | 471.00 | 9.76 | 0.18 | |
| | Asn | AAT | 314.00 | 6.51 | 0.22 | |
| | Asn | AAC | 1120.00 | 23.22 | 0.78 | |
| 40 | Met | ATG | 1077.00 | 22.32 | 1.00 | |
| | Ile | ATA | 88.00 | 1.82 | 0.05 | |
| | Ile | ATT | 315.00 | 6.53 | 0.18 | |
| | Ile | ATC | 1369.00 | 28.38 | 0.77 | |

| | | | | | |
|----|-----|-----|---------|-------|------|
| 5 | Thr | ACG | 405.00 | 8.40 | 0.15 |
| | Thr | ACA | 373.00 | 7.73 | 0.14 |
| | Thr | ACT | 358.00 | 7.42 | 0.14 |
| | Thr | ACC | 1502.00 | 31.13 | 0.57 |
| 10 | Trp | TGG | 652.00 | 13.51 | 1.00 |
| | End | TGA | 109.00 | 2.26 | 0.55 |
| | Cys | TGT | 325.00 | 6.74 | 0.32 |
| | Cys | TGC | 706.00 | 14.63 | 0.68 |
| 15 | End | TAG | 42.00 | 0.87 | 0.21 |
| | End | TAA | 46.00 | 0.95 | 0.23 |
| | Tyr | TAT | 360.00 | 7.46 | 0.26 |
| | Tyr | TAC | 1042.00 | 21.60 | 0.74 |
| 20 | Leu | TTG | 313.00 | 6.49 | 0.06 |
| | Leu | TTA | 76.00 | 1.58 | 0.02 |
| | Phe | TTT | 336.00 | 6.96 | 0.20 |
| | Phe | TTC | 1377.00 | 28.54 | 0.80 |
| 25 | Ser | TCG | 325.00 | 6.74 | 0.09 |
| | Ser | TCA | 165.00 | 3.42 | 0.05 |
| | Ser | TCT | 450.00 | 9.33 | 0.13 |
| | Ser | TCC | 958.00 | 19.86 | 0.28 |
| 30 | Arg | CGG | 611.00 | 12.67 | 0.21 |
| | Arg | CGA | 183.00 | 3.79 | 0.06 |
| | Arg | CGT | 210.00 | 4.35 | 0.07 |
| | Arg | CGC | 1086.00 | 22.51 | 0.37 |
| 35 | Gln | CAG | 2020.00 | 41.87 | 0.88 |
| | Gln | CAA | 283.00 | 5.87 | 0.12 |
| | His | CAT | 234.00 | 4.85 | 0.21 |
| | His | CAC | 870.00 | 18.03 | 0.79 |
| 40 | Leu | CTG | 2884.00 | 59.78 | 0.58 |
| | Leu | CTA | 166.00 | 3.44 | 0.03 |
| | Leu | CTT | 238.00 | 4.93 | 0.05 |
| | Leu | CTC | 1276.00 | 26.45 | 0.26 |
| 45 | Pro | CCG | 482.00 | 9.99 | 0.17 |
| | Pro | CCA | 456.00 | 9.45 | 0.16 |
| | Pro | CCT | 568.00 | 11.77 | 0.19 |
| | Pro | CCC | 1410.00 | 29.23 | 0.48 |

- According to a further aspect of the invention, an expression vector is provided which comprises and is capable of directing the expression of a polynucleotide sequence according to the first aspect of the invention, in particular wherein the codon usage pattern of the gp120 polynucleotide sequence is typical of highly expressed
- 5 mammalian genes, preferably highly expressed human genes. The vector may be suitable for driving expression of heterologous DNA in bacterial insect or mammalian cells, particularly human cells. In one embodiment, the expression vector is p7313 (see Figure 1).
- 10 In a further aspect the invention provides a method of treating or preventing HIV infections, any symptoms or diseases associated therewith, comprising administering a safe and effective amount of a polynucleotide, a vector, a polypeptide or a pharmaceutical composition according to the invention.
- 15 Administration of the pharmaceutical composition may take the form of one or of more than one individual doses, for example as repeat doses of the same DNA plasmid, or in a heterologous "prime-boost" vaccination regime, particularly a therapeutic vaccination regime. A heterologous prime-boost regime uses
- 20 administration of different forms of vaccine in the prime and the boost, each of which may itself include two or more administrations. Preferably but not necessarily the priming and boosting composition comprise the same antigens or different forms of the same antigens. The priming composition and the boosting composition will anyway have at least one antigen in common, although it is not necessarily an identical form of the antigen, it may be a different form of the same antigen. An
- 25 example of different forms of the same antigen is in the case of a polynucleotide encoding a gp120 which lacks a functional signal sequence and is substantially non-glycosylated in mammalian cells, and a polypeptide which is gp120 with its signal sequence and which is glycosylated. A full length and a truncated version of the same protein, or a mutated and a non-mutated form of the same protein, may also be
- 30 considered different forms of the same antigen for the purposes of a prime-boost format according to the invention.

In one example of a prime-boost regime the “prime” vaccination may be via particle mediated DNA delivery of a priming composition which comprises a polynucleotide or set of polynucleotides according to the present invention, preferably incorporated into a plasmid vector, while the “boost” administration may be of a boosting
5 composition comprising a recombinant viral vector comprising the same polynucleotide sequence or a polynucleotide encoding at least one of the same antigens encoded by the priming composition. Alternatively the boosting may be carried out with at least one of the same antigens in the form of the protein in adjuvant. Conversely the priming may be with a priming composition comprising the
10 viral vector or with a protein formulation typically a protein formulated in adjuvant, and the boost a DNA vaccine of the present invention.

A preferred prime-boost format for use with the polynucleotides according to the present invention is selected from:

- 15 Protein prime / live vector boost
- Live vector prime / protein boost
- Protein prime / DNA plasmid boost
- DNA plasmid prime / protein boost
- Live vector prime / DNA plasmid boost
- 20 DNA plasmid prime / live vector boost

Preferred live vectors include live virus vectors in particular adenovirus vectors as described herein.

25 Preferably the priming and boosting compositions comprise, in addition to the antigen(s) a suitable adjuvant, which may be different according to the particular composition.

Both the priming composition and the boosting composition may be delivered in more
30 than one dose. Furthermore the initial priming and boosting doses may be followed up with further doses which may be alternated to result in e.g. a DNA plasmid prime / protein boost / further DNA plasmid dose / further protein dose.

The invention further provides a process for the production of a polynucleotide as described herein comprising linking a nucleotide sequence encoding a substantially non-glycosylated HIV envelope molecule, preferably gp120 or fragment or immunogenic derivative thereof and optionally a sequence encoding an additional
5 HIV protein for example an HIV non-structural protein such as Tat or an HIV capsid protein such as Gag, or fragments or immunogenic derivatives thereof, to a heterologous promoter sequence.

As discussed above, the present invention includes expression vectors that comprise
10 the nucleotide sequences of the invention. Such expression vectors are routinely constructed in the art of molecular biology and may for example involve the use of plasmid DNA and appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals which may be necessary, and which are positioned in the correct orientation, in order to allow for protein expression. Other
15 suitable vectors and how to construct them would be apparent to persons skilled in the art. By way of further example in this regard we refer to Sambrook *et al.* Molecular Cloning: a Laboratory Manual. 2nd Edition. CSH Laboratory Press. (1989).

Preferably, a polynucleotide of the invention, or for use in the invention in a vector, is
20 operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence, such as a promoter, "operably linked" to a coding sequence is positioned in
25 such a way that expression of the coding sequence is achieved under conditions compatible with the regulatory sequence.

A nucleic acid sequence of the present invention may be administered by means of specialised delivery vectors useful in gene therapy. Gene therapy approaches are
30 discussed for example by Verme *et al.*, Nature 1997, 389:239-242. Both viral and non-viral vector systems can be used. The vectors may be, for example, plasmids, artificial chromosomes (e.g. BAC, PAC, YAC), virus or phage vectors provided with a origin of replication, optionally a promoter for the expression of the polynucleotide

and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin or kanamycin resistance gene in the case of a bacterial plasmid or a resistance gene for a fungal vector. Vectors may be used *in vitro*, for example for the production of DNA or RNA or used to transfect
5 or transform a host cell, for example, a mammalian host cell e.g. for the production of protein encoded by the vector. The vectors may also be adapted to be used *in vivo*, for example in a method of DNA vaccination or of gene therapy.

Examples of suitable viral vectors include retroviral, lentiviral, adenoviral, adeno-
10 associated viral, herpes viral including herpes simplex viral, alpha-viral, pox viral such as Canarypox and vaccinia-viral based systems. Gene transfer techniques using these viruses are known to those skilled in the art. Retrovirus vectors for example may be used to stably integrate the polynucleotide of the invention into the host genome, although such recombination is not preferred. Replication-defective
15 adenovirus vectors by contrast remain episomal and therefore allow transient expression. Vectors capable of driving expression in insect cells (for example baculovirus vectors), in human cells, yeast or in bacteria may be employed in order to produce quantities of the HIV protein encoded by the polynucleotides of the present invention, for example for use as subunit vaccines or in immunoassays.

20 In a preferred embodiment the adenovirus used as a live vector is a replication defective simian adenovirus. Typically these viruses contain an E1 deletion and can be grown on cell lines that are transformed with an E1 gene. Preferred Simian adenoviruses are viruses isolated from Chimpanzee. In particular C68 (also known as
25 Pan 9) (See US patent No 6083 716) and Pan 5, 6 and Pan 7 (W0 03/046124) are preferred for use in the present invention. Thus these vectors can be manipulated to insert a heterologous gene of the invention such that the gene product maybe expressed. The use, formulation and manufacture of such recombinant adenoviral vectors is set forth in detail in WO 03/046142.

30 Promoters and other expression regulation signals may be selected to be compatible with the host cell for which expression is designed. For example, mammalian promoters include the metallothionein promoter which can be induced in response to

heavy metals such as cadmium, and the β -actin promoter. Viral promoters such as the SV40 large T antigen promoter, human cytomegalovirus (CMV) immediate early (IE) promoter, rous sarcoma virus LTR promoter, adenovirus promoter, or a HPV promoter, particularly the HPV upstream regulatory region (URR) may also be used.

5 All these promoters are well described and readily available in the art.

A preferred promoter element is the CMV immediate early promoter devoid of intron A, but including exon 1. Accordingly there is provided a vector comprising a polynucleotide of the invention under the control of HCMV IE early promoter. A
10 suitable HCMV IE promoter is described in WO 02/36792.

Non-viral based systems include direct administration of nucleic acids, microsphere encapsulation technology, poly(lactide-co-glycolide) and liposome-based systems.

15 The polynucleotides according to the invention have utility in the production by expression of the encoded proteins, which expression may take place *in vitro*, *in vivo* or *ex vivo*. The nucleotides may therefore be involved in recombinant protein synthesis, for example to increase yields, or indeed may find use as therapeutic agents in their own right, utilised in DNA vaccination techniques. Where the polynucleotides
20 of the present invention are used in the production of the encoded proteins *in vitro* or *ex vivo*, cells, for example in cell culture, will be modified to include the polynucleotide to be expressed. Such cells include transient, or preferably stable mammalian cell lines. Particular examples of cells which may be modified by insertion of vectors encoding for a polypeptide according to the invention include
25 mammalian HEK293T, CHO, HeLa, 293 and COS cells. Preferably the cell line selected will be one which is stable. Expression may be achieved in transformed oocytes. A polypeptide may be expressed from a polynucleotide of the present invention, in cells of a transgenic non-human animal, preferably a mouse. A transgenic non-human animal expressing a polypeptide from a polynucleotide of the
30 invention is included within the scope of the invention.

The invention further provides a method of vaccinating a mammalian subject which

comprises administering thereto an effective amount of a vaccine or vaccine composition according to the invention. Preferably, expression vectors for use in DNA vaccines, vaccine compositions and immunotherapeutics will be plasmid vectors or live viral vectors.

5

DNA vaccines may be administered in the form of "naked DNA", for example in a liquid formulation administered using a syringe or high pressure jet, or DNA formulated with liposomes or an irritant transfection enhancer, or by particle mediated DNA delivery (PMDD or particle mediated immunotherapeutic delivery PMID) as described in more detail herein. All of these delivery systems are well known in the art. The vector may be introduced to a mammal for example by means of a viral vector delivery system.

The compositions of the present invention can be delivered by a number of routes such as intramuscularly, subcutaneously, intraperitoneally, intravenously or mucosally.

The invention further provides an intradermal delivery device comprising a pharmaceutical composition described herein.

In a preferred embodiment, the composition is delivered intradermally. In particular, the composition is delivered by means of a gene gun particularly using particle bombardment administration techniques which involve coating the vector on to beads (eg gold beads) which are then administered under high pressure into the epidermis. This is described, for example, in Haynes et al, J Biotechnology 44: 37-42 (1996).

25

Numerous methods of carrying out a particle bombardment approach are known, see for example WO 91/07487. In one illustrative example, gas-driven particle acceleration can be achieved with devices such as those manufactured by Powderject Pharmaceuticals PLC (Oxford, UK) and Powderject Vaccines Inc. (Madison, WI), some examples of which are described in U.S. Patent Nos. 5,846,796; 6,010,478; 5,865,796; 5,584,807; and EP Patent No. 0500 799. This approach offers a needle-free delivery approach wherein a dry powder formulation of microscopic particles, such as polynucleotide, are accelerated to high speed within a helium gas jet generated

30

by a hand held device, propelling the particles into a target tissue of interest, typically the skin. The particles are preferably gold beads of a 0.4 – 4.0 μm , more preferably 0.6 – 2.0 μm diameter and the DNA conjugate coated onto these and then encased in a cartridge or cassette for placing into the delivery device.

5

In a related embodiment, other devices and methods that may be useful for gas-driven needle-less injection of compositions of the present invention include those provided by Bioject, Inc. (Portland, OR), some examples of which are described in U.S. Patent Nos. 4,790,824; 5,064,413; 5,312,335; 5,383,851; 5,399,163; 5,520,639 and

10 5,993,412.

The vectors which comprise the nucleotide sequences encoding antigenic peptides are administered in such amount as will be prophylactically or therapeutically effective.

The quantity to be administered, is generally in the range of one picogram to 1
15 milligram, preferably 1 picogram to 10 micrograms for particle-mediated delivery, and 100 nanograms to 10 milligrams for other routes of nucleotide per dose. The exact quantity may vary considerably depending on the weight of the patient being immunised and the route of administration.

20 It is possible for the immunogen component comprising the nucleotide sequence encoding the antigenic peptide, to be administered on a one off basis or to be administered repeatedly, for example, between 1 and 7 times, preferably between 1 and 4 times, at intervals between about 1 day and about 18 months. Further administrations may also be given as necessary to maintain immune responses for the
25 lifetime of the patient. However, this treatment regime will be significantly varied depending upon the size of the patient concerned, the amount of nucleotide sequence administered, the route of administration, and other factors which would be apparent to a skilled medical practitioner. The patient may receive one or more other anti HIV retroviral drugs as part of their overall treatment regime. Additionally the nucleic acid
30 immunogen may be administered with an adjuvant.

The adjuvant component specified herein can similarly be administered via a variety of different administration routes, such as for example, via the oral, nasal, pulmonary, intramuscular, subcutaneous, intradermal or topical routes. Preferably, the adjuvant component is administered via the intradermal or topical route, most preferably by a topical route. This administration may take place between about 14 days prior to and about 14 days post administration of the nucleotide sequence, preferably between about 1 day prior to and about 3 days post administration of the nucleotide sequence.

The adjuvant component is, in one embodiment, administered substantially simultaneously with the administration of the nucleotide sequence. By “substantially simultaneous” what is meant is that administration of the adjuvant component is preferably at the same time as administration of the nucleotide sequence, or if not, it is at least within a few hours either side of nucleotide sequence administration. In the most preferred treatment protocol, the adjuvant component will be administered substantially simultaneously with administration of the nucleotide sequence. Obviously, this protocol can be varied as necessary, in accordance with the type of variables referred to above. It is preferred that the adjuvant is a 1H – imidazo [4,5c] quinoline – 4 – amine derivative such as imiquimod. Typically imiquimod will be presented as a topical cream formulation and will be administered according to the above protocol.

Once again, depending upon such variables, the dose of administration of the derivative will also vary, but may, for example, range between about 0.1 mg per kg to about 100 mg per kg, where “per kg” refers to the body weight of the mammal concerned. This administration of the 1H-imidazo[4,5-c]quinolin-4-amine derivative would preferably be repeated with each subsequent or booster administration of the nucleotide sequence. Most preferably, the administration dose will be between about 1 mg per kg to about 50 mg per kg. In the case of a “prime-boost” scheme as described herein, the imiquimod or other 1H-imidazo[4,5-c]quinolin-4-amine derivative may be administered with either the prime or the boost or with both the prime and the boost.

While it is possible for the adjuvant component to comprise only 1H-imidazo[4,5-c]quinolin-4-amine derivatives to be administered in the raw chemical state, it is preferable for administration to be in the form of a pharmaceutical formulation. That is, the adjuvant component will preferably comprise the 1H-imidazo[4,5-c]quinolin-4-amine combined with one or more pharmaceutically acceptable carriers, and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with other ingredients within the formulation, and not deleterious to the recipient thereof. The nature of the formulations will naturally vary according to the intended administration route, and may be prepared by methods well known in the pharmaceutical art. All methods include the step of bringing into association a 1H-imidazo[4,5-c]quinolin-4-amine derivative with an appropriate carrier or carriers. In general, the formulations are prepared by uniformly and intimately bringing into association the derivative with liquid carriers or finely divided solid carriers, or both, and then, if necessary, shaping the product into the desired formulation. Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a pre-determined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient.

Formulations for injection via, for example, the intramuscular, intraperitoneal, intradermal, or subcutaneous administration routes include aqueous and non-aqueous

sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. Formulations suitable for pulmonary administration via the buccal or nasal cavity are presented such that particles containing the active ingredient, desirably having a diameter in the range of 0.5 to 7 microns, are delivered into the bronchial tree of the recipient. Possibilities for such formulations are that they are in the form of finely comminuted powders which may conveniently be presented either in a piercable capsule, suitably of, for example, gelatine, for use in an inhalation device, or alternatively, as a self-propelling formulation comprising active ingredient, a suitable liquid propellant and optionally, other ingredients such as surfactant and/or a solid diluent. Self-propelling formulations may also be employed wherein the active ingredient is dispensed in the form of droplets of a solution or suspension. Such self-propelling formulations are analogous to those known in the art and may be prepared by established procedures. They are suitably provided with either a manually-operable or automatically functioning valve having the desired spray characteristics; advantageously the valve is of a metered type delivering a fixed volume, for example, 50 to 100 μL , upon each operation thereof.

25

In a further possibility, the adjuvant component may be in the form of a solution for use in an atomiser or nebuliser whereby an accelerated airstream or ultrasonic agitation is employed to produce a fine droplet mist for inhalation.

30

Formulations suitable for intranasal administration generally include presentations similar to those described above for pulmonary administration, although it is preferred for such formulations to have a particle diameter in the range of about 10 to about 200 microns, to enable retention within the nasal cavity. This may be achieved by, as

appropriate, use of a powder of a suitable particle size, or choice of an appropriate valve. Other suitable formulations include coarse powders having a particle diameter in the range of about 20 to about 500 microns, for administration by rapid inhalation through the nasal passage from a container held close up to the nose, and nasal drops
5 comprising about 0.2 to 5% w/w of the active ingredient in aqueous or oily solutions. In one embodiment of the invention, it is possible for the vector which comprises the nucleotide sequence encoding the antigenic peptide to be administered within the same formulation as the 1H-imidazo[4,5-c]quinolin-4-amine derivative. Hence in this embodiment, the immunogenic and the adjuvant component are found within the same
10 formulation.

In one embodiment the adjuvant component is prepared in a form suitable for biolistic administration, and is administered via that route substantially simultaneously with administration of the nucleotide sequence. For preparation of formulations suitable
15 for use in this manner, it may be necessary for the 1H-imidazo[4,5-c]quinolin-4-amine derivative to be lyophilised and adhered onto, for example, particles such as gold beads which are suited for biolistic administration.

In an alternative embodiment, the adjuvant component may be administered as a dry
20 powder, via high pressure gas propulsion.

Even if not formulated together, it may be appropriate for the adjuvant component to be administered at or about the same administration site as the nucleotide sequence.

25 Other details of pharmaceutical preparations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania (1985), the disclosure of which is included herein in its entirety, by way of reference.

Suitable techniques for introducing the naked polynucleotide or vector into a patient
30 also include topical application with an appropriate vehicle. The nucleic acid may be administered topically to the skin, or to mucosal surfaces for example by intranasal, oral, intravaginal or intrarectal administration. The naked polynucleotide or vector may be present together with a pharmaceutically acceptable excipient, such as

phosphate buffered saline (PBS). DNA uptake may be further facilitated by use of facilitating agents such as bupivacaine, either separately or included in the DNA formulation. Other methods of administering the nucleic acid directly to a recipient include ultrasound, electrical stimulation, electroporation and microseeding which is
5 described in US 5,697,901.

Uptake of nucleic acid constructs may be enhanced by several known transfection techniques, for example those including the use of transfection agents. Examples of these agents include cationic agents, for example calcium phosphate and DEAE-Dextran and lipofectants, for example lipofectam and transfectam. The dosage of the
10 nucleic acid to be administered can be altered.

A nucleic acid sequence of the present invention may also be administered by means of specialised delivery vectors useful in gene therapy. Gene therapy approaches are
15 discussed for example by Verme *et al*, Nature 1997, **389**:239-242. Both viral and non-viral vector systems can be used and are described above. Viral and non-viral delivery systems may be combined where it is desirable to provide booster injections after an initial vaccination, for example an initial "prime" DNA vaccination using a non-viral vector such as a plasmid followed by one or more "boost" vaccinations
20 using a viral vector or non-viral based system. Similarly the invention contemplates prime boost systems with the polynucleotide of the invention, followed by boosting with protein in adjuvant or vice versa.

A nucleic acid sequence of the present invention may also be administered by means
25 of transformed cells. Such cells include cells harvested from a subject. The naked polynucleotide or vector of the present invention can be introduced into such cells *in vitro* and the transformed cells can later be returned to the subject. The polynucleotide of the invention may integrate into nucleic acid already present in a cell by homologous recombination events. A transformed cell may, if desired, be
30 grown up *in vitro* and one or more of the resultant cells may be used in the present invention. Cells can be provided at an appropriate site in a patient by known surgical or microsurgical techniques (e.g. grafting, micro-injection, etc.)

The pharmaceutical compositions of the present invention may include adjuvant compounds as detailed above, or other substances which may serve to increase the immune response induced by the protein which is encoded by the DNA. These may be encoded by the DNA, either separately from or as a fusion with the antigen, or may be
5 included as non-DNA elements of the formulation. Examples of adjuvant-type substances which may be included in the formulations of the present invention include ubiquitin, lysosomal associated membrane protein (LAMP), hepatitis B virus core antigen, FLT3-ligand (a cytokine important in the generation of professional antigen presenting cells, particularly dendritic cells) and other cytokines such as IFN- γ and
10 GMCSF. Other preferred adjuvants include imiquimod and resiquimod and tucarasol, imiquimod being particularly preferred.

In a particular embodiment of the invention there is provided the use of a nucleic acid molecule as herein described for the treatment or prophylaxis of HIV infection,
15 administered with imiquimod. The imiquimod is preferably administered topically, whereas the nucleic acid molecule is preferably administered by means of particle mediated delivery.

Accordingly the present invention also provides a method of treating a subject
20 suffering from or susceptible to HIV infection, comprising administering a nucleic acid molecule as herein described and imiquimod.

The present invention will now be described by reference to the following examples:

EXAMPLES

Example 1: Plasmid Construction

5 1.1 Construction of gp120 containing plasmid

Recombinant gp120 glycoprotein described in the following examples is a synthetic form of the gp120 envelope protein of HIV-1 isolate W61D.

10 Codon Optimised (pgp120c):

The gene sequence was based on the gp120 sequence from the HIV-1 isolate W61D. This has a Codon Usage Coefficient of 0.297. Optimisation was performed using SynGene 2d, resulting in a CUC of 0.749 (Ertl, PF., Thomsen, LL. Technical issues in
15 construction of nucleic acid vaccines. (2003) Methods 31(3); 199-206. SynGene uses a mathematical method for codon optimisation based on the relative frequencies of use. Briefly, codons are assigned value ranges according to their frequencies, so that more frequent codons have wider ranges, and placed in ascending frequency order. The value ranges are expressed as ≥ 0.000 , $\geq 0.0??$, $\geq 0.???$ And so on. A
20 random number is generated between 0 and 0.99999. This is then used to select a codon, which will be the codon allocated the range within which the random number falls. To exclude rare codons the value 0.1 is added to the random number, so that it falls in the range 0.1-1.09999.

25 The gp120 sequence was split into 40 overlapping oligonucleotides, PCR assembled and recovered using the end primers. The gene was cloned into vector p7313-ie (shown in Figure 1) as a NotI-BamHI fragment and sequenced. Restriction fragments from three initial clones were combined to generate a single correct clone. The amino acid sequence and codon optimised DNA sequence are given in Figure 2.

30

1.2 Generation of Nef/Tat containing plasmids

Nef/Tat (pNTm and ptrNTm)

The gene for the Nef/Tat fusion protein was provided in plasmid pRIT15244 (Figure 3). The plasmid pRIT 15244 is identical to pRIT 14913 described below except that the His tail has been deleted.

5 General

The Nef gene from the Bru/Lai isolate (Cell 40: 9-17, 1985) was selected for the constructs since this gene is among those that are most closely related to the consensus Nef.

10

The starting material for the Bru/Lai Nef gene was a 1170bp DNA fragment cloned on the mammalian expression vector pcDNA3 (pcDNA3/Nef).

15 The Tat gene originates from the BH10 molecular clone. This gene was received as an HTLV III cDNA clone named pCV1 and described in Science, 229, p69-73, 1985. This *tat* gene bears mutations in the active site region (Lys41→Ala) and in RGD motif (Arg78→Lys and Asp80→Glu) (Virology 235: 48-64, 1997).

20 The mutant *tat* gene was received as a cDNA fragment subcloned between the EcoRI and HindIII sites within a CMV expression plasmid (pCMVLys41/KGE)

Construction of vector pRIT14597 (encoding Nef-His protein).

25 The *nef* gene was amplified by PCR from the pcDNA3/Nef plasmid with primers 01 and 02.

NcoI

PRIMER 01: 5'ATCGTCCATG.GGT.GGC.AAG.TGG.T 3' [SEQ ID NO: 1]

30

SpeI

PRIMER 02: 5' CGGCTACTAGTGCAGTTCTTGAA 3' [SEQ ID NO:2]

The integrative vector PHIL-D2 (INVITROGEN) was used. This vector was modified in such a way that expression of heterologous protein starts immediately after the native ATG codon of the AOX1 gene and will produce recombinant protein with a tail of one glycine and six histidines residues. This PHIL-D2-MOD vector was constructed by cloning an oligonucleotide linker between the adjacent *AsuII* and *EcoRI* sites of PHIL-D2 vector. In addition to the His tail, this linker carries *NcoI*, *SpeI* and *XbaI* restriction sites between which *nef*, *tat* and *nef-tat* fusion were inserted.

The *nef* PCR fragment obtained and the integrative PHIL-D2-MOD vector were both restricted by *NcoI* and *SpeI*, purified on agarose gel and ligated to create the integrative plasmid pRIT14597.

Construction of vector pRIT14913 (encoding fusion Nef-Tat mutant-His).

To construct pRIT14913, the *tat* mutant gene was amplified by PCR from the pCMVLys41/KGE plasmid with primers 03 and 04.

SpeI

PRIMER 03: 5' ATCGTACTAGT.GAG.CCA.GTA.GAT.C 3' [SEQ ID NO: 3]

SpeI

PRIMER 04: 5' CGGCTACTAGTTTTCCTTCGGGCCT 3' [SEQ ID NO: 4]

The PCR fragment obtained and the plasmid pRIT14597 (expressing Nef-His protein) were both digested by *SpeI* restriction enzyme, purified on agarose gel and ligated to create the integrative plasmid pRIT14913.

1.3 Generation of PMID vectors for gp120 and Nef/Tat:

gp120: Codon-optimised gp120 was provided as described above.

5 **Nef/Tat (pNTm and ptrNTm):**

The gene for the Nef/Tat fusion protein was provided in plasmid pRIT15244 described above. The Tat in this plasmid contains three mutations to inactivate the transactivation function. The fusion contains full length Nef which has an immune
10 modulatory function (Collins and Baltimore (1999)) that may be abrogated by N-terminal truncation. Therefore constructs were generated for both full length Nef/mutant Tat(pNTm) and truncated Nef/mutant Tat(ptrNTm), in which the first 65 amino acids of Nef were removed. These sequences were PCR amplified from pRIT15244 using primers:

15

5'Nef GAATTCGCGGCCCGCCATGGGTGGCAAGTGGTCAAAAAG

5'trNef GAATTCGCGGCCCGCCATGGTGGGTTTTCCAGTCACACC

3'Tat GAATTCGGATCCTTATTCCTTCGGGCCTGTCGGG

[SEQ ID NOS: 5,6,7]

20

The genes were cloned into vector p7313-ie as NotI-BamHI fragments and sequenced. PNTm and ptrNTm and the Nef/Tat and truncated Nef/Tat sequences are shown in Figures 4 and 5.

25 **Dual expression vectors: (pRIX1 and pRIX2)**

The Nef/Tat and trNef/Tat expression cassettes were excised as ClaI-XmnI restriction fragments, and ligated into the ClaI and blunted Sse8387 I sites of the vector containing the codon optimised gp120 (pgp120c) to provide single plasmids for
30 expression of both proteins (pRIX1 and pRIX2 respectively).

Composition of plasmid p7313-ie (Figure 1)

The plasmid was constructed by replacing the beta-lactamase gene containing Eam1105I - Pst1 fragment of pUC19 (available from Amersham Pharmacia Biotech UK Ltd., Amersham Place, Little Chalfont, Bucks, HP7 9NA) with an EcoRI fragment of pUC4K (Amersham-Pharmacia) containing the Kanamycin resistance gene, following blunt ending of both fragments using T4 DNA polymerase. The human Cytomegalovirus IE1 promoter /enhancer, Intron A, was derived from plasmid JW4303 obtained from Dr Harriet Robinson, University of Massachusetts, and inserted into the Sal1 site of pUC19 as a XhoI -Sal1 fragment, incorporating the bovine growth hormone polyadenylation signal. Deletion of the 5' SalI-BanI fragment from the promoter generated the minimal promoter used in the vector (WO00/23592 - Powderject Vaccines Inc.). HBV Surface antigen 3'UTR was derived from Hepatitis B Virus, serotype adw, in the vector pAM6 (Moriarty et al., Proc.Natl.Acad.Sci. USA, 78, 2606-10, 1981). pAM6 (pBR322 based vector) was obtained from the American Type Culture Collection, catalogue number ATCC 45020. The 3'UTR was inserted 5' to the polyadenylation signal as a 1.4kb BamHI fragment, blunt ended for insertion to remove the BamHI sites. In a series of steps (including digestion with *Bgl* II, Klenow polymerase treatment, digestion with *Bst*X I, digestion with *Nco* I, treatment with mung bean nuclease to remove overhang and further digestion with *Bst*X I), modifications were made to the region between the 3'untranslated enhancer region of the HBV S gene and bGHpA signal to remove all open reading frames of greater than 5 codons between the X gene promoter and the bGHpA signal. This resulted in deletion of sequence encoding the translatable portion of the X protein (9 amino acids) and the X gene start codon. The bovine growth hormone polyadenylation signal was substituted with the rabbit beta globin polyadenylation signal. The 5'non-coding and coding sequences of the S antigen were excised and replaced with an oligonucleotide linker to provide multiple cloning sites as shown to produce plasmid p7313-PL.

30

Hind---NotI--- EcoRV- -NdeI- -BamHI
 AGCTTGCGGCCGCTAGCGATATCGGTACCATATGTCGACGGATCC....
ACGCCGGCGATCGCTATAGCCATGGTCTACAGCTGCCTAGGCCGG

-NheI- -KpnI- -SalI- ΔNotI

[SEQ ID NO: 8]

This polylinker was further extended by insertion of an additional oligonucleotide
5 linker between the KpnI and SalI sites:

AspI- -MunI- NaeI- NdeI-- BglII-
GTACCGGTCAATTGGCGCCGGCGCGCCATATGACGTCAGATCTG-----
-----GCCAGTTAACCGCGGCGCGCGGTATACTGCAGTCTAGACAGCT
10 --AgeI- -NarI-- AatII- SalI
[SEQ ID NO: 9]

The ColE1 cer sequence was obtained from a subclone from plasmid pDAH212 from
David Hodgeson (Warwick University) and amplified by PCR using primers to place
15 EcoRI restriction sites at the ends of the sequence. The cer sequence was then inserted
into the EcoRI site of p7313-PL to produce plasmid p7313-PLc. The sequence of the
amplified cer was verified against the Genbank entry M11411.

The HBV 3'UTR sequence between the promoter and polyadenylation signal was
removed by PCR amplification of the polyadenylation signal using primers:
20 sense: CCATGGATCCGATCTTTTCCCTCTGCC [SEQ ID NO: 10]
antisense: GTTAGGGTGAAAAGCTTCCGAGTGAGAGACAC [SEQ ID NO: 11]

The resulting product was cut with BamHI and XmnI and used to replace the
corresponding fragment containing both the polyadenylation signal and the 3'UTR.
The Intron A sequence was removed from the plasmid by PCR amplification of the
25 CMV promoter/enhancer using primers:

sense: GCTAGCCTGCAGGCTGACCGCCCAACGAC [SEQ ID NO: 12]
antisense: GTTCTCCATCGCGGCCGCACTCTTGGCACGGGG [SEQ ID NO: 13]

The resulting product was cut with Sse8387 I and NotI, and inserted back into the
Sse8387 I and NotI sites of the parental vector.

30

Example 2: Modification of gp120 and Nef/Tat(mut) expression vectors

gp120 constructs were modified to reduce secretion of the protein.

Generation of constructs:

gp120 without a secretion signal (dsgp120, pRix 12 – see Figure 2 and 6)

5

The gp120 gene was PCR amplified from pgp120c using the following primers:

5'120ds: 5'GAATTCGCGGCCCGCCATGGCCGAGCAGCTGTGGGTCACC
[SEQ ID NO: 14]

10 L01:

5'GAATTCGGATCCTCATCTCTGCACGACGCGGCGCTTGGCCCGGGT
GGGGGCCACG [SEQ ID NO: 15]

Fragments were amplified using PWO DNA polymerase (Roche) and the cycle:

15

95°C (30s) 95°C(30s) 50°C(30s) 72°C(90s) 72°C(120s) 4°C(hold)
____ repeat x20 ____/

20 The products were cut with NotI and BamHI and cloned into p7313-ie to give pRix12
(Figure 6).

Results

25 In 293T cells the vector pRIX12, which lacks the secretion signal, makes a good
amount of a 60kDa non-glycosylated protein that is not secreted (Figures 29 and 30).

30 **Example 3: Construction of vectors for expression of gp120 and Nef/Tat(mut)**
from a single plasmid

Vector construction:

The gp120 Nef/Tat(m) constructs were generated by PCR stitching the gp120 and Nef/Tat(m) or trNef/Tat(m) orfs.

5' and 3' Gp120, 5' and 3' Nef/Tat(m) and 5'trNef/Tat were amplified from pRix1.

5 3'trNef/Tat(m) was amplified from pNTm. The following primers were used:

3'120: (antisense to): GCCAAGCGCCGCGTCGTGCAGAGA [SEQ ID NO: 16]

5'120/NT:

GCCAAGCGCCGCGTCGTGCAGAGAATGGGTGGCAAGTGGTCAAAAAGT

10 [SEQ ID NO: 17]

3'NT (antisense to): GGGGAGCCGACAGGCCCGAAGGAA [SEQ ID NO: 18]

5'NT/120:

GGGGAGCCGACAGGCCCGAAGGAAATGAAGGTCAAGGAGACCAGAAAG

15 [SEQ ID NO: 19]

5'120/trN:

GCCAAGCGCCGCGTCGTGCAGAGAATGGTGGGTTTTCCAGTCAC

[SEQ ID NO: 20]

5'trNef:

20 GAATTCGCGGCCGCCATGGTGGGTTTTCCAGTCACACC [SEQ ID
NO: 21]

L01:

GAATTCGGATCCTCATCTCTGCACGACGCGGCGCTTGGCCCCGGGTG
GGGGCCACG [SEQ ID NO: 22]

25

L02:

ACCACCTTGTA CTTGTACAGCTCGCTCCGCCAGTTATCCCTCATGTC
GCCGCCGCCGGGC [SEQ ID NO: 23]

30

Fragments were amplified using PWO DNA polymerase (Roche) and the cycle:

95°C (60s) 95°C(30s) 55°C(30s) 72°C(120s) 72°C(120s) 4°C(hold)

_____ repeat x20 _____/

Primer L1 was used as the 3' primer for 3'gp120. However there were problems using this primer when stitching Nef/Tat or trNef/T to the 5' end of gp120 so primer L2 was
5 used instead.

The stitched gp120-N/Tm and gp120-trN/Tm fragments were cut with NotI and BamHI and cloned into similarly cut p7313-*ie*. Due to the use of primer L2 rather than L1 the N/Tm-gp120 and trN/Tm-gp120 fragments lacked a BamHI site, so these were
10 cut with NotI and AccI, and cloned into similarly cut pgp120c. All inserts were fully verified by sequencing. The plasmids were designated pRix6 (gp120c NefTat^m) and pRix11 (gp120c trNefTat^m).

15 **Example 4: Construction of vectors to investigate the effects of glycosylation and secretion, inclusion of Tat and inclusion of Gag (p17/24) and Nef and RT on gp120 and gp120 fusions**

Vectors were constructed as shown in Figure 29 and 30 (schematic).

20 pRix 28 and pRix29 (Figures 7 and 8)

pRix28 and 29 containing ds gp120c NefTat^m and ds gp120c trNefTat were generated by transferring the AccI-BamHI fragments from pRix6 (2315bp) and pRix11 (2123bp)
25 into similarly cut pRix12 (ds gp120c).

pRix30 and pRix31 (Figure 9)

To generate glycosylated and non-glycosylated fusion vectors of gp120c Nef without
30 Tat, the NotI-KpnI fragment was transferred from pRix11 (1580bp) or pRix29 (1496bp) into similarly cut pRix15, a vector containing Tat/trNef.

(pRix15) - Tat(mut)trNef

The genes for Tat and trNef were PCR amplified from pNTm using the following primers:

- 5 5'Tat: 5'GAATTCGCGGCCGCCATGGAGCCAGTAGATCCTAGAC [SEQ ID NO: 24]
 3'Tat: 5'TTCCTTCGGGCCTGTCGGC [SEQ ID NO: 25]
 5'trTN: GCCGACAGGCCCGAAGGAAATGGTGGGTTTCCAGTCACAC [SEQ ID
 NO: 26]
 3'Nef: GAATTCGGATCCTTAGCAGTTCTTGAAGTACTCCGG [SEQ ID NO: 27]

10

The individual genes were gel purified and then PCR stitched to give TmtrN using the end primers. The fusion was then digested with NotI and BamHI and cloned into p7313-ie.

15 pRix32

To generate the fusion containing p17/24, gp120 was PCR amplified from pgp120c using primers U1 and 3'120, p17/24-Nef was amplified from p73i-GN2 using primers 5'120G and 3'Nef, and the two were PCR stitched using U1 and 3'Nef. p73I-GN2
 20 contained a synthetic codon optimised sequence of p17/p24 based on the sequence of HXB2 (GenBank entry K03455) and designed using SynGene and assembled from overlapping oligonucleotides as described for codon optimised gp120 above, fused to HXB2 Nef, which had been obtained from plasmid pHXBΔPr (B.Maschera, E Furfine and E.D. Blair 1995 J.Virol 69 5431-5436) by PCR. Since the HXB2 nef gene in this
 25 plasmid contains a premature termination codon two overlapping PCRs were used to repair the codon (TGA [stop] to TGG [Trp]). The position of the repaired codon is underlined in the sequence. The p17/p24/Nef gene was inserted into the NotI and BamHI sites of plasmid p7313ie. The coding sequence and map is given in Figure 10. A * marks the p24/trNef junction.

30

Primers:

U1:

GAATTCGCGGCCGCAATGAAGGTCAAGGAGACCAGAAAGAACTACCAGC
ATCTGTG [SEQ ID NO: 28]

3'120: TCTCTGCACGACGCGGCGCTTGGC [SEQ ID NO: 29]5'120G:

5 GCCAAGCGCCGCGTCGTGGAGAGAATGGGTGCCCCGAGCTTCGGTAC [SEQ
ID NO: 30]

3'Nef: GAATTCGGATCCTTAGCAGTTCTTGAAGTACTCCGG [SEQ ID NO: 31]

Initial cycle:

10 94°C(30s) 20x[94°C (30s) 50°C (30s) 68°C (180s)] 68°C (120s) 4°C (0s)

Using pfx polymerase with 1x enhancer.

Stitch:

94°C(30s) 20x[94°C (30s) 50°C (30s) 72°C (180s)] 72°C (120s) 4°C (0s)

15 Using Vent polymerase in standard conditions + 2mM MgCl₂.

The product was cut with NotI and BamHI and cloned into p7313-ie.

On sequencing the construct was found to have a error in the signal peptide, which
20 was corrected by transferring the 2560bp BstEII - KpnI fragment containing the back
of gp120 to the front of Nef into pRix30.

pRix 33 (Figure 11), pRix34, and pRix35 (Figure 12)

25 The 2560bp BstEII - KpnI fragment containing the back of gp120 to the front of Nef
was transferred to pRix31, pRix11 and pRix29 to make vectors pRix 33 (Figure 11),
34 and 35 (Figure 12) respectively.

pRix39 (gp120 codon optimised, minus secretion signal - p17/24 gag - Nef-Tat –
30 Figure 13) and pRix40-47 (Constructs pRix 40-47 contain non-glycosylated gp120,
gag-p17/24, Nef and Tat(m) fusions with mutations in the miristoylation site and/or
dileucine motif of Nef)

A fragment containing gag p17/24 was PCR amplified from vector pRix35 using primers:

5'120G:

5 GCCAAGCGCCGCGTCGTGGAGAGAATGGGTGCCCGAGCTTCGGTAC [SEQ ID NO: 32]

and

p24AS: CAACACTCTGGCTTTGTGTCC [SEQ ID NO: 33]

10 Full length Nef was PCR amplified from pNTm using primers:

5'p24-N: GGACACAAAGCCAGAGTGTTGATGGGCAAGTGGTCAAAAAGTAG [SEQ ID NO: 34]

and

3'Nef: GAATTCGGATCCTTAGCAGTTCTTGAAGTACTCCGG [SEQ ID NO: 35]

15

The two fragments were PCR stitched together using the end primers (5'120G and 3'Nef). The product was cut with Sall and KpnI, and the 423bp fragment containing part of p24 and Nef was used to replace the corresponding fragment in pRix35 to make pRix39 (Figure 13).

20

pRix40 (Figure 14) was similarly constructed except primer 5'p24-N was replaced with primer:

5'p24-Ndm:

25 GGACACAAAGCCAGAGTGTTGATGGGCAAGTGGTCAAAAAGTAG [SEQ ID NO: 36]

G H K A R V L | M G K W S K S

This primer deletes the one G to destroy the miristoylation site at the start of Nef.

30 pRix41-44, 46 and 47 (Figures 15 to 20)

Mutations to the dileucine motif in Nef (L174L175) were made by PCR:

To insert the mutations, the portion of Nef 5' to the LL motif was PCR amplified using the 5'Nef primer and asNefLL

5'Nef GAATTCGCGGCCGCCATGGGTGGCAAGTGGTCAAAAAG [SEQ ID
5 NO: 37]

asNefLL (Antisense to)

GCCAATAAAGGAGAGAACACCAGC [SEQ ID NO: 38]

A N K G E N T S

10 Mutations to L174, L175 or both 174 and 175 were generated using forward primers

sNefL1 (L174A)

GCCAATAAAGGAGAGAACACCAGCGCCTTACACCCTGTGAGCCTGCATG

A N K G E N T S | A L H P V S L H

15 [SEQ ID NO: 39]

sNefL2 (L175A)

GCCAATAAAGGAGAGAACACCAGCTTGGCACACCCTGTGAGCCTGCATG

A N K G E N T S | L a H P V S L H

20 [SEQ ID NO: 40]

SnefLL (LL174/5AA)

GCCAATAAAGGAGAGAACACCAGCGCCGCACACCCTGTGAGCCTGCATG

A N K G E N T S | A A H P V S L H

25 [SEQ ID NO: 41]

and the 3'NT primer:

3'NT (antisense to):GGGGAGCCGACAGGCCCGAAGGAA [SEQ ID NO: 42]

30

to amplify the 3' portion of Nef. The 5' and each of the 3' products were PCR stitched using the 5'Nef and 3'NT primers. These were cut with KpnI and SpeI and inserted into similarly cut pRix39 to generate pRix41 (L174A), pRix42 (L175A), and pRix43 (LL174/175A) in the absence of the myristoylation site mutation, or into pRix40 to

generate pRix44 (mLL174/175AA) pRix46 (mL174A) and pRix47 (mL175A) with the myristoylation site mutation.

pRix50, 51 and 52 and 53, 54 and 60 (Figures 23 to 27)

- 5 To generate dual promoter vectors containing non-glycosylated gp120 initially pRix12 was cut with Sse8387I, blunt ended with T4 DNA polymerase to remove the overhangs, and cut with ClaI. The 4573bp product was gel purified (Fragment A)

pRix50: the plasmid pNTm was cut with ClaI and XmnI and the 1897bp fragment was
10 gel purified and ligated with Fragment A.

pRix51: the plasmid p73i-GN2 was cut with ClaI and XmnI and the 2533bp fragment was gel purified and ligated with Fragment A.

- 15 pRix52: Plasmids p73i-GN2 and pNTm were cut with BglII and EcoRI, and the 4090bp and 1060bp fragments were isolated. From the respective digests. These fragments were ligated to produce the vector pRix52.

pRix53: the plasmid pRix52 was cut with ClaI and XmnI and ApaLI and the 2794bp
20 fragment was gel purified and ligated with Fragment A.

pRix54: the plasmid pT-rng (described in PG5035) was cut with ClaI and XmnI and the 4219bp fragment was gel purified and ligated with Fragment A.

- 25 pRix60: pT-rng was cut with Sse8387I, blunt ended with T4 DNA polymerase to remove the overhangs, and cut with ClaI. The large fragment was gel purified. The plasmid pRix12 was cut with ClaI and XmnI and DraI and the 2476bp fragment was gel purified and ligated with the fragment from pT-rng.

30 pRix58 (Figure 21)

The gp120 fragment without signal sequence was PCR amplified from pgp120c using the primers:

5' ds120: GAATTCGCGGCCGCCATGGCCGAGCAGCTGTGGGTCACC [SEQ ID NO: 43]

3'120: (antisense to): GCCAAGCGCCGCGTCGTGCAGAGA [SEQ ID NO: 44]

5

the 5' end of RT (codon optimised and containing the W229K inactivating mutation) was PCR amplified from pt-rng (Figure 28 – see also WO 03/025003) using a 5' primer to insert a sequence homologous to 3'120, and a primer within RT

10 120RTf: GCCAAGCGCCGCGTCGTGCAGAGAATGGGCCCCATCAGTCCCATC [SEQ ID NO: 45]

RT3SR1: CGTCACGATGTTACCTCCAGGCC [SEQ ID NO: 46]

The two products were PCR stitched using the end primers, and cut with NotI and
15 NheI. The fragment was gel purified and used to replace the NotI -NheI fragment from pT-rng.

pRix59 (Figure 22): the 3'Gag fragment was PCR amplified from pT-rng using a primer 5' to the MunI site in p24 and a 3' primer encoding the start of ds120,
20 covering the position of the BstEII site near the 5' end:

GagMunf

GTGGCCCGAGAGCTGCATCCG [SEQ ID NO: 47]

25 GAG120R (Antisense to:)

Gag-----ds120

GGACACAAAGCCAGAGTGTTGATGGCCGAGCAGCTGTGGGTCACCGTC
[SEQ ID NO: 48]

30 The product was cut with MunI and BstEII, and inserted into the 7113bp fragment from MunI-BstEII cut pRix54.

Results

Expression data is shown in Figures 29, 30 and 31.

5 293T cell monolayers in 24 well plates were transfected with 1 µg of each DNA indicated using Lipofectamine 2000 following the manufacturer's supplied protocol. After 24 hours the cells were detached and separated from the culture medium by centrifugation. Samples equivalent to 1×10^4 cells or 12 µl of medium were examined by PAGE and Western blot.

10

The gp120c construct gave a highly glycosylated well secreted protein. Addition of c-terminal Nef/Tat fusions (pRix 6 and pRix11) resulted in a reduction of the intracellular protein levels and loss of secretion. Removal of the secretion signal from gp120c (pRix12) gave a non-glycosylated non-secreted form of the protein.

15

As expected, fusion constructs with no secretion signal pRix28, 29, 31, 33 and 35, made non-glycosylated intracellular proteins in similar amounts, though expression from pRix35 was somewhat reduced. Surprisingly, when the secretion signal was present in constructs pRix30, 32 and 34, only pRix34 failed to be secreted. It appears
20 that the presence of Tat in the fusion inhibits secretion of the protein. The initial pRix32.1 construct had a point mutation resulting in poor expression. This was corrected in pRix32.7, which showed greatly improved expression.

25

For pRix40-47 the western blot in Figure 31 shows the expression of the ds-gp120/Gag/Nef/Tat fusions with mutations in Nef in 293T cells 24 hours post transfection with the plasmids indicated. Total cell extracts equivalent to $\sim 1 \times 10^4$ cells were loaded onto the gel. The blot was probed with an anti-nef antiserum.

30

For pRix50, 51, 53, 54, 58, 59 and 60 the two sets of western blots in Figure 31 show the expression of the products of the dual promoter vectors indicated. Total cell extracts equivalent to $\sim 1 \times 10^4$ 293T cells, 24 hours post transfection, were loaded onto the gel. The left blot was probed with an anti-nef antiserum, and the right blot was probed with anti-gp120 antiserum.

Refs:

Andre S. Seed B. Eberle J. Schraut W. Bultmann A. Haas J. (1998) Increased
5 immune response elicited by DNA vaccination with a synthetic gp120 sequence with
optimized codon usage. Journal of Virology. 72(2):1497-503.

Vinner L. Nielsen HV. Bryder K. Corbet S. Nielsen C. Fomsgaard A. (1999) Gene
gun DNA vaccination with Rev-independent synthetic HIV-1 gp160 envelope gene
10 using mammalian codons. Vaccine. 17(17):2166-75

Collins KL. Baltimore D.(1999) HIV's evasion of the cellular immune response.
Immunological Reviews. 168:65-74.

15

**Example 5: Preparation of plasmid-coated 'gold slurry' for 'gene gun' DNA
cartridges**

Plasmid DNA (approximately 1 µg/µl), eg. 100 ug, and 2 µm gold particles, eg. 50 mg,
20 (PowderJect), were suspended in 0.05M spermidine, eg. 100 ul, (Sigma). The DNA
was precipitated on to the gold particles by addition of 1M CaCl₂, eg. 100ul
(American Pharmaceutical Partners, Inc., USA). The DNA/gold complex was
incubated for 10 minutes at room temperature, washed 3 times in absolute ethanol, eg.
3 x 1 ml, (previously dried on molecular sieve 3A (BDH)). Samples were resuspended
25 in absolute ethanol containing 0.05mg/ml of polyvinylpyrrolidone (PVP, Sigma), and
split into three equal aliquots in 1.5 ml microfuge tubes, (Eppendorf). The aliquots
were for analysis of (a) 'gold slurry', (b) eluate- plasmid eluted from (a) and (c) for
preparation of gold/ plasmid coated Tefzel cartridges for the 'gene gun', (see Example
3 below). For preparation of samples (a) and (b), the tubes containing plasmid DNA /
30 'gold slurry' in ethanol / PVP were spun for 2 minutes at top speed in an Eppendorf
5418 microfuge, the supernatant was removed and the 'gold slurry' dried for 10
minutes at room temperature. Sample (a) was resuspended to 0.5 – 1.0 ug / ul of
plasmid DNA in TE pH 8.0, assuming approx. 50 % coating. For elution, sample (b)

was resuspended to 0.5 – 1.0 ug / ul of plasmid DNA in TE pH 8.0 and incubated at 37°C for 30 minutes, shaking vigorously, and then spun for 2 minutes at top speed in an Eppendorf 5418 microfuge and the supernatant, eluate, was removed and stored at -20°C. The exact DNA concentration eluted was determined by spectrophotometric
5 quantitation using a Genequant II (Pharmacia Biotech).

Example 6: Preparation of Cartridges for DNA immunisation

Preparation of cartridges for the Accell gene transfer device was as previously
10 described (Eisenbraun et al DNA and Cell Biology, 1993 Vol 12 No 9 pp 791-797; Pertner et al). Briefly, plasmid DNA was coated onto 2 µm gold particles (DeGussa Corp., South Plainfield, N.J., USA) and loaded into Tefzel tubing, which was subsequently cut into 1.27 cm lengths to serve as cartridges and stored desiccated at 4°C until use. In a typical vaccination, each cartridge contained 0.5 mg gold coated
15 with a total of 0.5 µg DNA/cartridge.

Example 7: PMID immunisations including using gp120-Nef-Tat triple fusion lacking gp120 secretion signal

20

Protocol: For PMID immunisations (DNA) cartridges were prepared for using standard methods as described in Examples 5 and 6. A DNA loading rate of 2, which will give approximately 0.5 µg DNA/cartridge was used and each immunisation consisted of two shots. Balb/c mice were given a primary immunisation of DNA
25 (using PMID). The mice were boosted 28 days later with DNA (using PMID). Mice were culled 7 days later and serum and spleens were collected. The splenocytes were harvested by teasing out the spleen cells and erythrocytes were lysed. The splenocytes were washed and counted. Specialised ELIspot plates (coated with interferon-gamma capture antibody and blocked) were used. Splenocytes were transferred to these plates
30 and incubated overnight at 37°C/5% CO₂ in the presence of a gp120 peptide, RT peptide or Gag peptide. The splenocytes were lysed and the plate developed using standard procedures to demonstrate the number of interferon-gamma secreting cells

present. Serum was analysed by ELISA assay to detect for specific antibodies. Results are shown in Figures 32-34.

Conclusion

5 Unexpectedly, the cellular immune response of mice immunised with dsGP120 (gp120 lacking secretion signal) expressing constructs was approximately double that of mice immunised with gp120 constructs (see Figure 32 and 33). This was consistent with the observation that in *in vitro* transfection studies the expression of dsGP120 had remained largely cell associated, whereas gp120 had been excreted.

10

Inclusion of Tat (mutated Tat) in the dsGP120 constructs increased the cellular immune response to twice that of the dsGP120 constructs without Tat (Figures 32 and 33). Tat on its own did not affect the immune response to gp120, but acted synergistically with dsGP120 to optimise the cellular response.

15

The inclusion of other HIV antigens in the constructs or dual promoter vectors produced a balanced cellular response to all the different antigens included and thus broadened the immune response compared to the gp120 only vectors (Figure 34).

20

CLAIMS

1. A polynucleotide which comprises a sequence encoding an HIV envelope
5 protein or fragment or immunogenic derivative thereof, which is substantially non-
glycosylated when expressed in a mammalian target cell, operably linked to a
heterologous promoter.
2. The polynucleotide according to claim 1 wherein the HIV envelope protein or
10 fragment or immunogenic derivative thereof is gp120 or a fragment or immunogenic
derivative thereof.
3. The polynucleotide according to claim 1 or claim 2 wherein the envelope
protein lacks a functional secretion signal.
- 15 4. The polynucleotide according to claim 2 or claim 3 wherein the gp120 is
expressed as a fusion protein comprising at least one other HIV protein or fragment or
immunogenic derivative thereof.
- 20 5. The polynucleotide according to claim 4 wherein the at least one other HIV
protein or fragment or immunogenic derivative is selected from Nef, Gag, RT or Tat.
6. The polynucleotide according to claim 5 wherein the gp120 encoding sequence
is linked to a sequence encoding HIV RT or a fragment or immunogenic derivative
25 thereof and a sequence encoding HIV Gag or a fragment or immunogenic derivative
thereof and a sequence encoding HIV Nef or a fragment or immunogenic derivative
thereof to encode a gp120, RT, Gag and Nef-containing fusion protein.
7. The polynucleotide according to claim 6 wherein the fusion is selected from
30 gp120-RT-Nef-Gag and RT-Nef-Gag-gp120.

8. The polynucleotide according to claim 5 wherein the gp120 sequence is linked to a sequence encoding HIV Tat or a fragment or immunogenic derivative thereof and a sequence encoding HIV Nef or a fragment or immunogenic derivative thereof to encode a gp120, Nef and Tat-containing fusion protein.
- 5
9. The polynucleotide according to claim 8 wherein the fusion is a gp120-Nef-Tat fusion.
10. The polynucleotide according to claim 8 wherein the gp120 is further linked to a sequence encoding HIV Gag or a fragment or immunogenic derivative thereof to encode a gp120, Nef, Tat and Gag-containing fusion protein.
- 10
11. The polynucleotide according to claim 10 wherein the fusion is a gp120-Gag-Nef-Tat fusion.
- 15
12. The polynucleotide according to any one of claims 5, 6, 7, 10 or 11 wherein the Gag comprises P17 and/or 24.
13. The polynucleotide according to any one of claims 1 to 12 wherein one or more of the sequences encoding gp120, Nef, Gag, RT or Tat or fragment or immunogenic derivative thereof is or are codon optimised to resemble the codon usage in a highly expressed human gene.
- 20
14. A polynucleotide sequence selected from the group:
- 25
1. gp120 codon optimised, minus secretion signal
 2. gp120 codon optimised, minus secretion signal – tr Nef
 3. gp120 codon optimised, minus secretion signal – tr Nef – mTat
 4. gp120 codon optimised, minus secretion signal – Nef - mTat
 - 30 5. gp120 codon optimised, minus secretion signal – p17/24 Gag – tr Nef
 6. gp120 codon optimised, minus secretion signal – p17/24 Gag – tr Nef - mTat
 7. gp120 codon optimised, minus secretion signal - p17/24 Gag - Nef-mTat
 8. gp120 codon optimised, minus secretion signal - p17/24 Gag - mNef-mTat

9. gp120 codon optimised, minus secretion signal - p17/24 Gag - L1Nef-mTat
10. gp120 codon optimised, minus secretion signal - p17/24 Gag - L2Nef-mTat
11. gp120 codon optimised, minus secretion signal - p17/24 Gag - LLNef-mTat
12. gp120 codon optimised, minus secretion signal - p17/24 Gag - mLLNef-mTat
- 5 13. gp120 codon optimised, minus secretion signal - p17/24 Gag - mL1Nef-mTat
14. gp120 codon optimised, minus secretion signal - p17/24 Gag - mL2Nef-mTat
15. gp120 codon optimised, minus secretion signal - mRT- trNef - p17/24 Gag
16. mRT – trNef – p17/24 Gag – gp120 codon optimised, minus secretion signal

Wherein the RT and Gag are codon optimised.

10

15. The polynucleotide according to any one of claims 1 to 14 wherein the promoter is the promoter from HCMV IE gene.

16. The polynucleotide according to claim 15 wherein the 5' untranslated region
15 between the promoter and coding polynucleotide comprises exon 1.

17. A set of polynucleotides comprising a polynucleotide according to any of claims 1 to 16 and at least one further polynucleotide encoding at least one of HIV Nef, Gag, RT or Tat or fragment or immunogenic derivative thereof.

20

18. The set of polynucleotides according to claim 17 wherein the polynucleotides are contained on a single vector under the control of two or more separate promoters.

19. The set of polynucleotides according to claim 17 or 18, encoding a gp120 or
25 fragment or immunogenic derivative and a fusion of RT-Nef-Gag or of fragments or immunogenic derivatives thereof.

20. A set of polynucleotides according to claim 17 or claim 18 selected from the following:

- 30 1. gp120 codon optimised, minus secretion signal + tr Nef-mTat
2. gp120 codon optimised, minus secretion signal + P17/24 Gag - tr Nef
3. gp120 codon optimised, minus secretion signal + P17/24 Gag - Nef - mTat
4. mRT – tr Nef – P17/24 Gag + gp120 codon optimised, minus secretion signal

5. gp120 codon optimised, minus secretion signal + mRT – tr Nef – P17/24 Gag wherein RT and Gag are codon optimised.
21. A vector comprising a polynucleotide or polynucleotides as claimed in any one
5 of claims 1 to 20.
22. The vector according to claim 21 which is a double stranded DNA plasmid.
23. The vector according to claim 21 which is a replication defective adenovirus
10 vector.
24. The vector according to claim 23 which is derived from Pan 9, 5, 6 or 7.
25. A fusion protein comprising a substantially non-glycosylated HIV envelope
15 protein or a fragment or immunogenic derivative thereof and at least one additional HIV protein or fragment or immunogenic derivative thereof said additional HIV protein selected from Nef, Gag, RT and Tat.
26. A composition comprising a substantially non-glycosylated HIV envelope
20 protein or a fragment or immunogenic derivative thereof and at least one additional HIV protein or fragment or immunogenic derivative thereof, preferably in the form of a fusion protein, said at least one additional HIV protein selected from Nef, Gag, RT and Tat.
27. A polypeptide encoded by the polynucleotide or vector according to any of
25 claims 1 to 24.
28. A pharmaceutical composition comprising a nucleotide sequence or a set of nucleotide sequences according to any one of claims 1 to 20, a vector of claim 21 to
30 24, a fusion protein according to claim 25 or a composition according to claim 26 or a polypeptide according to claim 27, and a pharmaceutically acceptable excipient, diluent, carrier or adjuvant.

29. The pharmaceutical composition according to claim 28 wherein the carrier is a plurality of particles such as gold beads.
30. The pharmaceutical composition according to claim 28 or 29 for delivery in a prime boost format.
31. An intradermal delivery device comprising a pharmaceutical composition according to any one of claims 28 to 30.
32. A method of treating a patient suffering from or susceptible to a disease comprising administering a safe and effective amount of a pharmaceutical composition according to any one of claims 28 to 30.
33. A polynucleotide or a vector or fusion protein or composition or polypeptide according to any one of claims 1 to 27 for use in medicine.
34. Use of a polynucleotide or a vector or fusion protein or composition or polypeptide according to any one of claims 1 to 27 in the manufacture of a medicament for the treatment of disease.
35. A process for the production of a polynucleotide according to any one of claim 1 to 20 comprising linking a nucleotide sequence encoding a substantially non-glycosylated HIV envelope molecule or fragment or immunogenic derivative thereof and optionally a sequence encoding an HIV regulatory protein or fragment or immunogenic derivative thereof, to a heterologous promoter sequence.

Figure 1

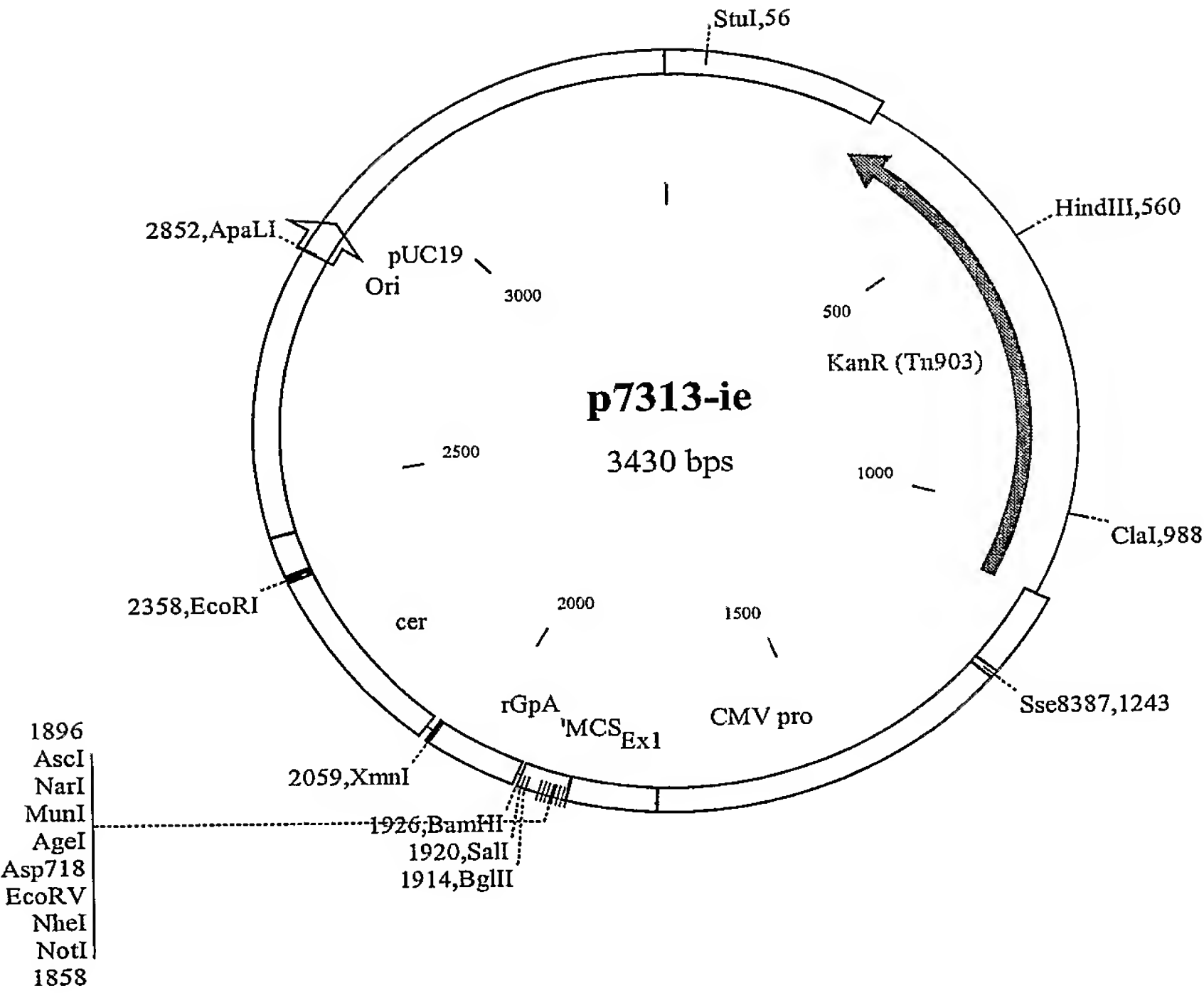
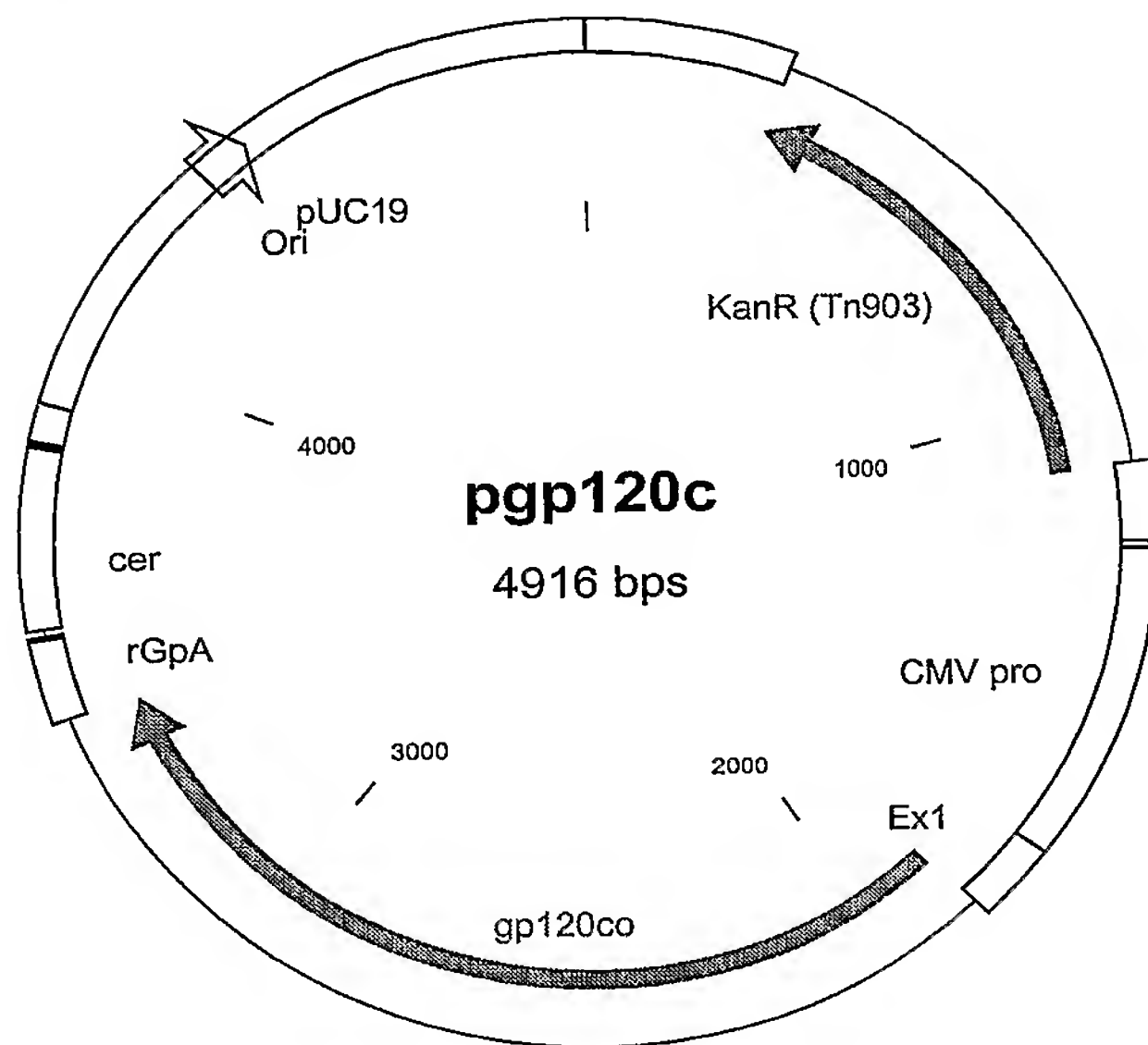


Figure 2

Map of pgp120c:



The amino acid sequence of the W61D gp120 is below. The signal sequence is underlined and bold, up to the predicted cleavage site between amino acids 29 and 30. This is the sequence removed in dsGP120 (pRix12 etc).

MKVKETRKNYQHLWRWGTMLLGMLMICSAAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNWATH
 ACVPTDPNPQEVVLGNVTEYFNMWKNMVDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTT
 SNGWTGEIRKGEIKNCSEFNITTSIRDKVQKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQA
 CPKVSFEPIPIHYCAPAGFAILKCNNKTFDGKGLCTNVSTVQCTHGIRPVVSTQLLNGSLAESEEVVIRSD
 NFMNNTKTIIVQLNESVAINCTRPNNNTRKGIHIGPGRAFYAARKIIGDIRQAHCNLSRAQWNNTLQKQIVI
 KLREHFGNKTIFKNQSSGGDPEIVRHSFNCGGEFFYCDTTQLFNSTWNGTEGNNTTEGNSTITLPCRICKQII
 NMWQEVGKAMYAPPIGGQIRCSSNITGLLLTRDGGTEGNGTENETEIFRPGGGDMRDNWRSELYKYKVVKV
 EPLGVAPTRAKRRVVQR [SEQ ID NO: 49]

The codon optimised DNA sequence for the W61D gp120 gene is:

ATGAAGGTCAAGGAGACCAGAAAGAACTACCAGCATCTGTGGCGCTGGGGCACCATGCTCCTGGGAATGCT
 GATGATCTGCTCCGCCGCCGAGCAGCTGTGGGTCACCGTCTACTACGGCGTGCTGTGTGGAAGGAGGCCA
 CGACCACCTCTTCTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCAT
 GCTTGCGTGCTTACGGACCCCAACCCCAAGAGGTGGTGCTGGGAAACGTGACCGAGTACTTCAACATGTG
 GAAGAATAACATGGTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCG
 TGAAGCTGACGCCTCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACC
 AGCAACGGCTGGACCGGAGAGATTCGGAAGGGCGAGATCAAGAAGTCTCCTTCAATATCACGACCTCGAT
 CAGAGACAAGGTGCAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATG
 CCACCACCAAGAACAAGACGACGCGTAATTTTCAGACTCATTCACTGCAACAGCAGCGTCATGACGCGAGGCC
 TGCCCCAAGGTGTCTTTCGAACCAATCCCGATCATTACTGTGCCCCCTGCCGGATTCGCGATCCTCAAGTG
 TAACAACAAGACCTTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCC

Figure 2 continued

GCCCCGTCGTGAGCACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGATCCGGTCGGAC
AACTTCATGGACAACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCC
TAACAACAACACCCGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCCGCAAGATCATCG
GCGACATCCGGCAGGCCCATTGCAACCTCTCCCGCGCCCAGTGGAATAACACCCTGAAGCAGATCGTGATC
AAGCTGAGAGAGCACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGT
GCGGCACTCCTTCAACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGA
ACGGCACCGAGGGCAACAACACAGAGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATC
AACATGTGGCAGGAGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAA
CATCACCGGCCTGCTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCT
TCAGGCCCGGCGGCGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTG
GAGCCGCTCGGCGTGGCCCCCACCCGGGCCAAGCGCCGCGTCGTGCAGAGATGA [SEQ ID NO: 50]

Figure 3

Map of pRix15244:

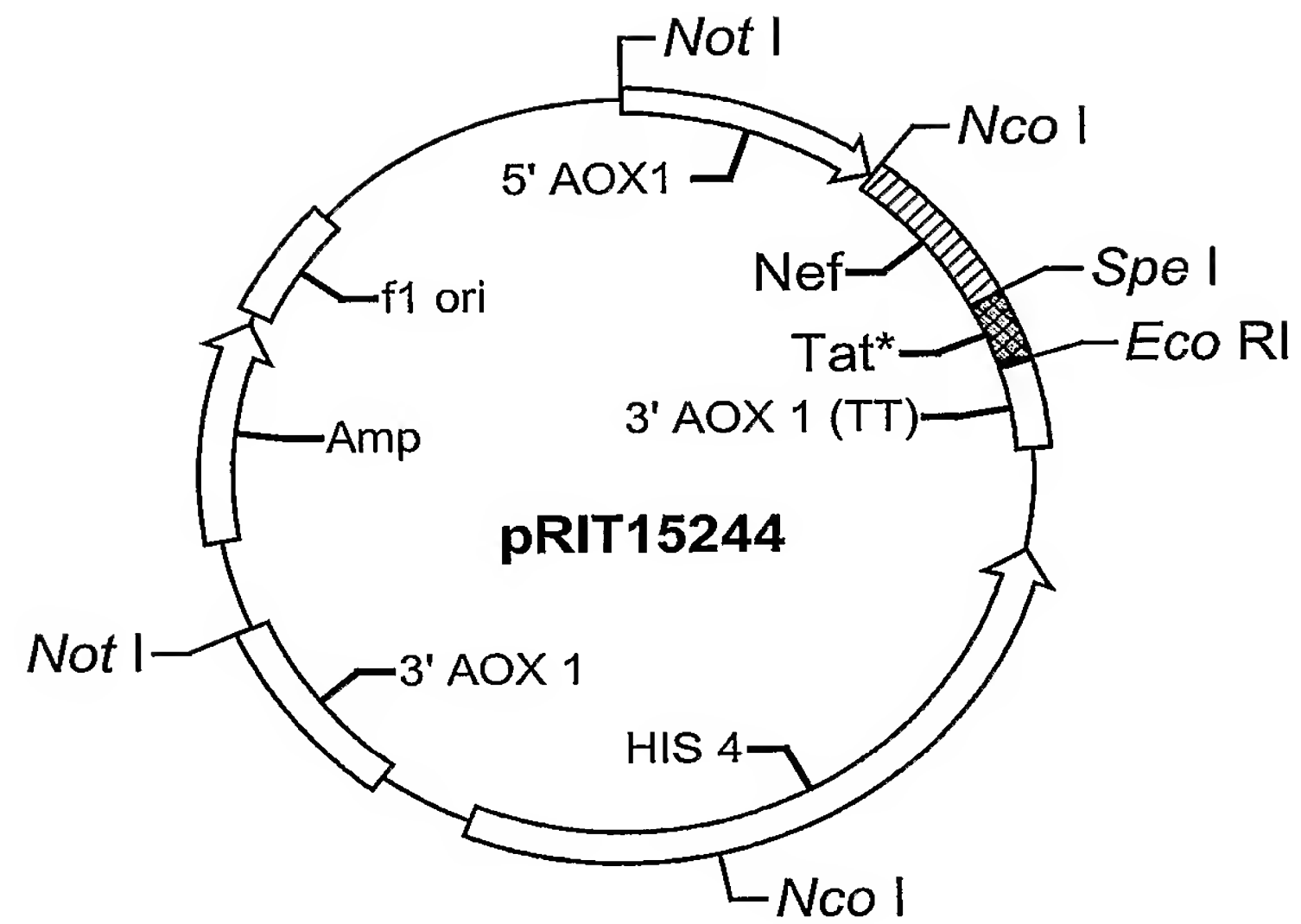
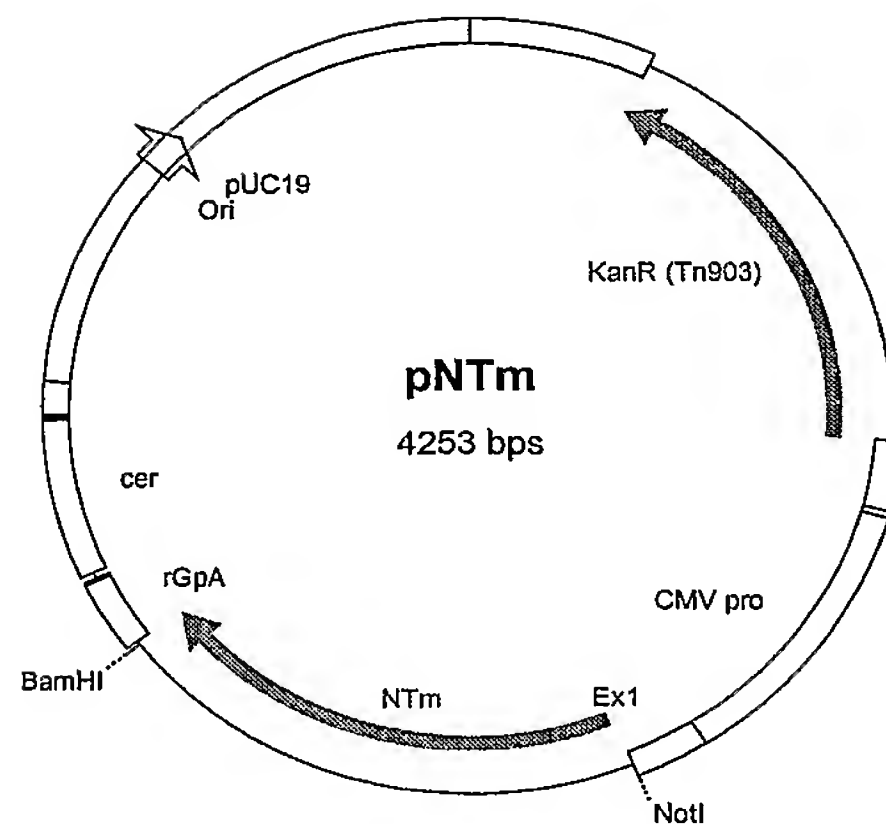


Figure 4

Plasmid pNTm:



Sequence of insert:

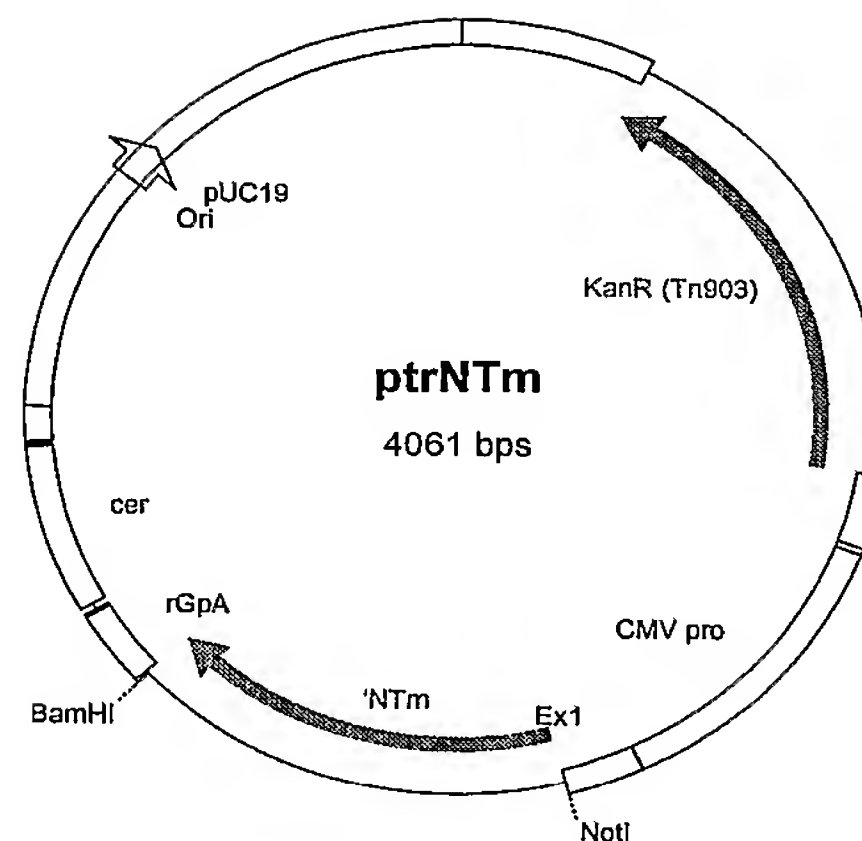
ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGA
GCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACAAGTAGCAATA
CAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACA
CCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGG
GGGACTGGAAGGGCTAATTCACCTCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAG
GCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTTGGATGGTGC
TACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACA
CCCTGTGAGCCTGCATGGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGAGAGTTTGACAGCCGCCTAG
CATTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCT
AGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACCTGCTTGTACCAATTGCTATTGTAAAAAGTG
TTGCTTTCATTGCCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGAC
AGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCC
AAAGGGGAGCCGACAGGCCCGAAGGAATAA [SEQ ID NO: 51]

Amino acid sequence of antigen:

MGGKWSKSSVVGWPTVRRMRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQEE
 EEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHTQGYFPDWQNYT
 PPGVRYPLTFGWICYKLVPVEPDKVEEANKGENTSLLHPVSLHGMDDPEREVLEWRFD SRLAFH
 HVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITAALGISYGRK
 KRRQRRRPPQGSQTHQVSLSKQPTSQSKGEPTGPKE [SEQ ID NO: 52]

Figure 5

Plasmid ptrNTm:



Sequence of insert:

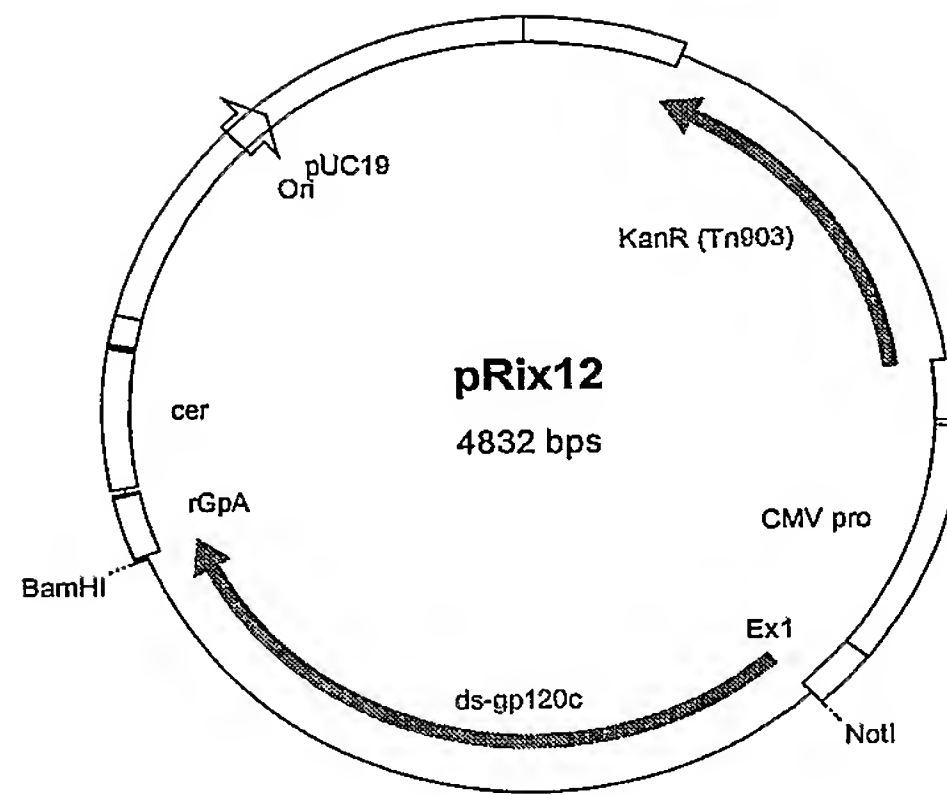
ATGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAG
 CCACTTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACCTCCAACGAAGACAAGATATCCTTGATC
 TGTGGATCTACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATAT
 CCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGG
 AGAGAACACCAGCTTGTTACACCTGTGAGCCTGCATGGAATGGATGACCCTGAGAGAGAAGTGTTAGAGT
 GGAGGTTTGACAGCCGCCTAGCATTTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGC
 ACTAGTGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAAGTCTTGTAC
 CAATTGCTATTGTAAAAAGTGTGCTTTTCATTGCCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCT
 ATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCA
 AAGCAACCCACCTCCCAATCCAAAGGGGAGCCGACAGGCCCGAAGGAATAA [SEQ ID NO: 53]

Amino acid sequence of antigen:

MVGFVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHTQGYFPDWQNYTPGPGVRY
 PLTFGWCYKLVPVEPDKVVEANKGENTSLHPVSLHGMDPEREVLEWRFD SRLAFHHVARELHPEYFKNC
 TSEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLS
 KQPTSQSKGEPTGPKE [SEQ ID NO: 54]

Figure 6

Plasmid pRix12:



Sequence of insert:

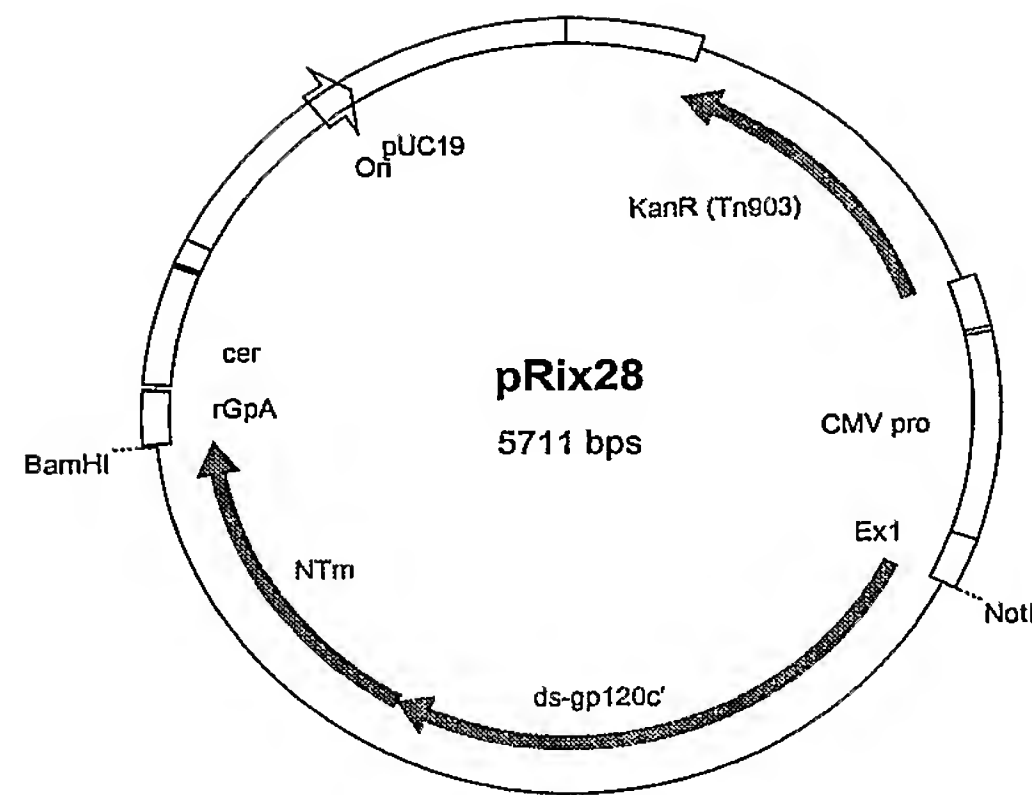
ATGGCCGAGCAGCTGTGGGTCACCGTCTACTACGGCGTGCTGTGTGGAAGGAGGCCACGACCACCTCTT
 CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
 CGGACCCCAACCCCAAGGAGGTGGTGTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTCTGGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTTCAGACTCATTCCTGCAACAGCAGCGTCATGACGCAGGCCTGCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCTGCGGATTGCGCGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCCGCCCCGTGCTGAG
 CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGTATCCGGTCGGACAACCTTCATGGACA
 ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCGCAAGATCATCGGCGACATCCGGCA
 GGCCCATTGCAACCTCTCCCGCGCCAGTGGAATAACACCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCGAGGG
 CAACAACACAGAGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTG
 CTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCCACC CGGCCAAGCGCCGCTCGTGCAGAGATGA [SEQ ID NO: 55]

Amino acid sequence of antigen:

MAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVVLGNVTEYFNMWKNM
 VDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTTSSNGWTGEIRKGEIKNCSFNITTSIRDKV
 QKEYALFYNLDVVPIDDDNATTKNKTTTRNFRLIHCNSSVMTQACPKVSFEPPIPIHYCAPAGFAILKCNKNT
 FDGKGLCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEVVRSDNFMDNTKTIIVQLNESVAINCTRPNNT
 RKGIIHIGPGRAFYAARKIIGDIRQAHCNLSRAQWNNTLKQIVIKLREHFGNKTIFNQSSGGDPEIVRHSF
 NCGGEFFYCDTTQLFNSTWNGTEGNNTGNSTITLPCRIKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
 LLTRDGGTEGNGTENETEIFRPGGGDMRDNRSELYKYKVVKEPLGVAPTRAKRRVVQR [SEQ ID
 NO: 56]

Figure 7

Plasmid pRix28:



Sequence of insert:

ATGGCCGAGCAGCTGTGGGTACCGTCTACTACGGCGTGCCTGTGTGGAAGGAGGCCACGACCACCCTCTT
 CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
 CGGACCCCAACCCCCAGGAGGTGGTGTCTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTCTGGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTAGACTCATTCACTGCAACAGCAGCGTCATGACGCGAGGCCTGCCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCTGCGGATTCGCGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGACGTGCACCCATGGCATCCGCCCCGTGCTGAG
 CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGATCCGGTCGGACAACCTTCATGGACA
 ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCGCAAGATCATCGGCGACATCCGGCA
 GGCCCATTGCAACCTCTCCCGCGCCCAAGTGGAAATAACCCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCGAGGG
 CAACAACACAGAGGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTG
 CTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCCACCCGGGCCAAGCGCCGCGTCGTGCAGAGAATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTT
 GGATGGCCTACTGTAAGGGGAAAGAATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCG
 AGACCTGGAAAAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCCTGGCTAG
 AAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAG
 GCAGCTGTAGATCTTAGCCACTTTTTTAAAGAAAAGGGGGGACTGGAAGGGCTAATTCCTCCCAACGAAG
 ACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAG
 GGCCAGGGGTCAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTA
 GAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTACACCTGTGAGCCTGCATGGAATGGATGACCTGA
 GAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCTAGCATTTTCATCACGTGGCCCGAGAGCTGCATCCGG
 AGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAG

Figure 7 continued

CCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCATTGCCAAGTTTGTTTCATAACAGC
TGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTC
ATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCAAAGGGGAGCCGACAGGCCCGAAGGAATAA

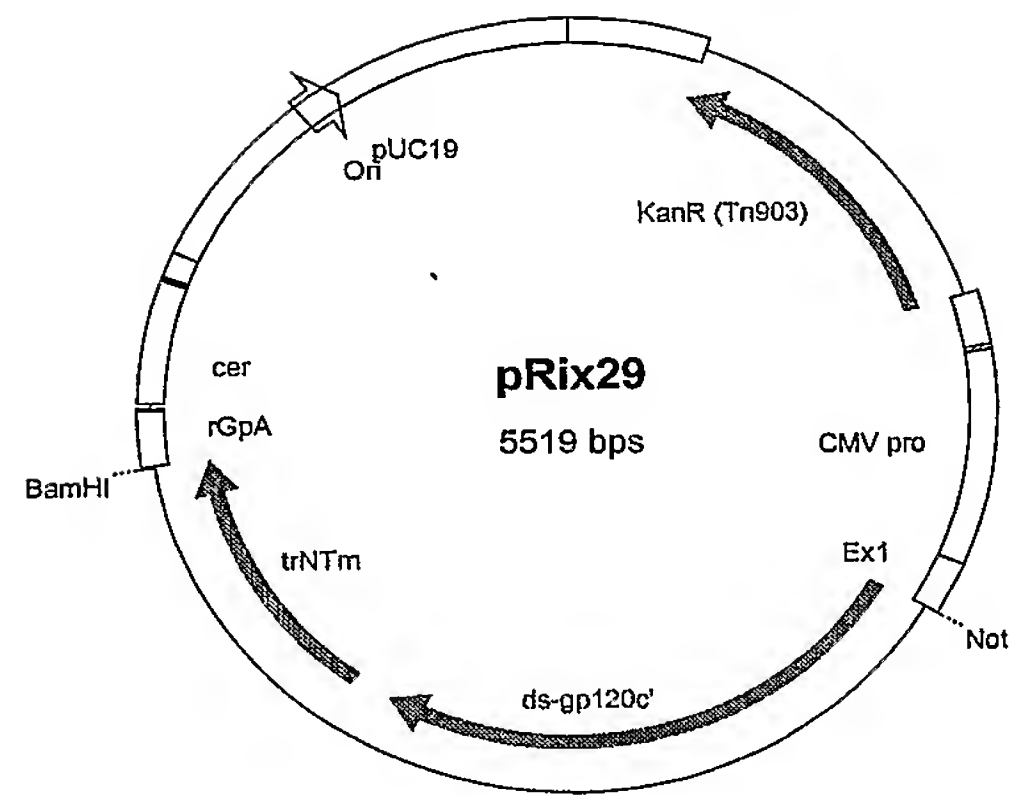
[SEQ ID NO: 57]

Amino acid sequence of antigen:

MAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEVVLGNVTEYFNMWKNM
VDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTTSNGWTGEIRKGEIKNCSEFNITTSIRDKV
QKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNNKT
FDGKGLCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSDNFMNDNTKTIIVQLNESVAINCTRPNNNT
RKGIHIGPGRAFYAARKIIGDIRQAHCNLSRAQWNNTLKQIVIKLREHFGNKTIKFNQSSGGDPEIVRHSF
NCGGEFFYCDTTQLFNSTWNGTEGNNTGNSTITLPCRIKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
LLTRDGGTEGNGTENETEIFRPGGGDMRDNRSELYKYKVVKVEPLGVAPTRAKRRVVQRMGGKWSKSSVV
GWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQEEEEVGFPVTPQVPLRPMTYK
AAVDLSHFLKEKGGLEGLIHSQRRQDILDWYHTQGYFPDWQNYTPGPGVRYPLTFGWICYKLVPVEPDKV
EEANKGENTSLLHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQ
PKTACTNCYCKKCCFHCQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSKGEPTGPKE
[SEQ ID NO: 58]

Figure 8

Plasmid pRix29:



Sequence of insert:

ATGGCCGAGCAGCTGTGGGTCACCGTCTACTACGGCGTGCTGTGTGGAAGGAGGCCACGACCACCCTCTT
 CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
 CGGACCCCAACCCCAAGGAGGTGGTGTCTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTCTGGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTTCACTCATTCACTGCAACAGCAGCGTCATGACGCAGGCCCTGCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCTTGCCGGATTCGCGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCCGCCCCGTGCTGAG
 CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGATCCGGTCCGACAACCTTCATGGACA
 ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCGCAAGATCATCGGCGACATCCGGCA
 GGCCCATTTGCAACCTCTCCCGCGCCAGTGGAATAACACCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCGAGGG
 CAACAACACAGAGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTG
 CTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCCACCCGGGCCAAGCGCCGCGTCTGTGACAGAGAATGGTGGGTTTTCCAGTCACACCTCAGGTACCT
 TTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAGAAAAGGGGGGACTGGAAGG
 GCTAATTCCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTG
 ATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTA
 CCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCTGTGAGCCT
 GCATGGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCTAGCATTTTCATCACG
 TGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTAGAGCCC
 TGGAAGCATCCAGGAAGTCAGCCTAAAACCTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTTCATTG
 CCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGAC
 CTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCAAAGGGGAGCCG
 ACAGGCCCGAAGGAATAA [SEQ ID NO: 59]

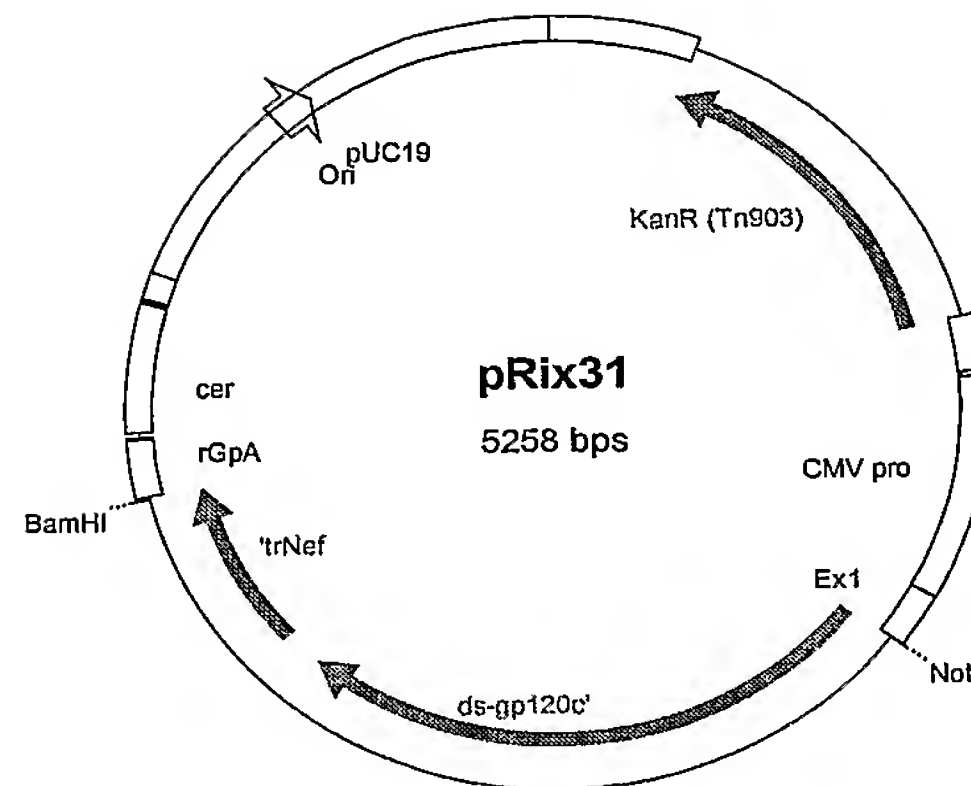
Figure 8 continued

Amino acid sequence of antigen:

MAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEVVLGNVTEYFNMWKNNM
VDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTTSNGWTGEIRKGEIKNCSEFNITTSIRDKV
QKEYALFYNLDVVPIDDDNATTKNKTRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNNKT
FDGKGLCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSDNFMDNTKTIIVQLNESVAINCTRPNNNT
RKGIIHIGPGRAFYAARKIIGDIRQAHCNLSRAQWNNTLKQIVIKLREHFGNKTIKFNQSSGGDPEIVRHSF
NCGGEFFYCDTTQLFNSTWNGTEGNNTGNSTITLPCRIKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
LLTRDGGTEGNGTENETEIFRPGGGDMRDNRSELYKYKVVKVEPLGVAPTRAKRRVVQRMVGFVPVTPQVP
LRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLV
PVEPDKVEEANKGENTSLHHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEP
WKHPGSQPKTACTNCYCKKCCFHCQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSKGEP
TGPKE [SEQ ID NO: 60]

Figure 9

Plasmid pRix31:



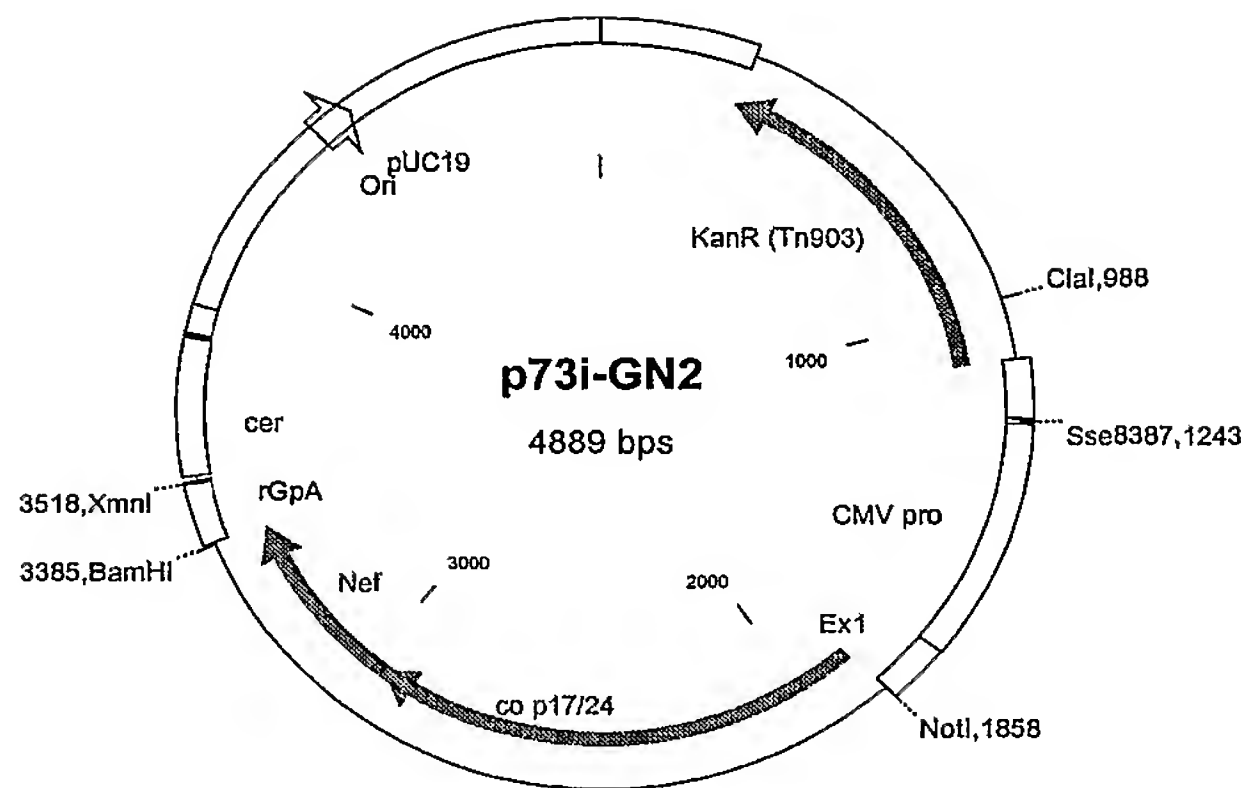
Sequence of insert:

ATGGCCGAGCAGCTGTGGGTCACCGTCTACTACGGCGTGCCTGTGTGGAAGGAGGCCACGACCACCCTCTT
 CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
 CGGACCCCAACCCCAAGGAGGTGGTGTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTGCGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTTCAGACTCATTCACTGCAACAGCAGCGTCATGACGCGAGGCCTGCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCTGCGGATTCGCGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCCGCCCCGTCGTGAG
 CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGATCCGGTCGGACAACCTTCATGGACA
 ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCTTCTATGCCGCCCAGATCATCGGCGACATCCGGCA
 GGCCCATTTGCAACCTCTCCCGCGCCAGTGGAATAACACCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCGAGGG
 CAACAACACAGAGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTG
 CTGCTCACCAAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCCACCCGGGCCAAGCGCCGCGTCGTGCAGAGAATGGTGGGTTTTCCAGTCACACCTCAGGTACCT
 TTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTAAAGAAAAGGGGGGACTGGAAGG
 GCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTG
 ATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTA
 CCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCT
 GCATGGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCTAGCATTTTCATCAG
 TGGCCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCTAA [SEQ ID NO: 61]

Amino acid sequence of antigen:

Figure 9 continued

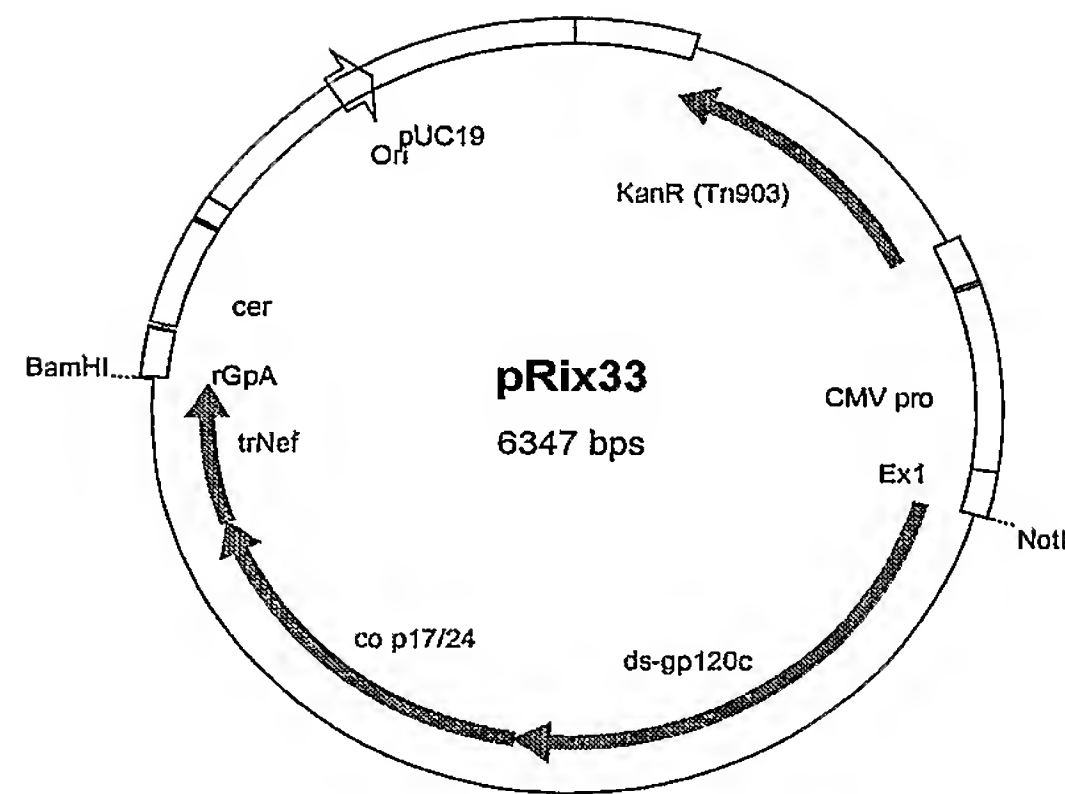
MAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEVVLGNVTEYFNMWKNNM
VDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTTSNGWTGEIRKGEIKNCSFNITTSIRDKV
QKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNNKT
FDGKGLCTNVSTVQCTHGIRPVVSTQLLLNGSLAEFEVIRSDNFMDNTKTIIVQLNESVAINCTRPNNNT
RKGIIHIGPGRAFYAARKIIGDIRQAHCNLSRAQWNNTLKQIVIKLREHFGNKTIKFNQSSGGDPEIVRHSF
NCGGEFFYCDTTQLFNSTWNGTEGNNTGNSTITLPCRICKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
LLTRDGGTEGNGTENETEIFRPGGGDMRDNRSELYKYKVVKVEPLGVAPTRAKRRVVQRMVGFPVTPQVP
LRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHTQGYFPDWQNYTPGPGVRYPLTFGW CYKLV
PVEPDKVEEANKGENTSLLHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNC
[SEQ ID NO: 62]

Figure 10**Sequence of insert:**

TGGGTGCCCCGAGCTTCGGTACTGTCTGGTGGAGAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCCGGGA
 GGCAAAAAGAAATACAAGCTCAAGCATATCGTGTGGGCCTCGAGGGAGCTTGAACGGTTTGCCGTGAACCC
 AGGCCTGCTGGAAACATCTGAGGGATGTCGCCAGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGA
 GTGAAGAGCTGAGGTCCTTGTATAACACAGTGGCTACCCTCTACTGCGTACACCAGAGGATCGAGATTAAG
 GATACCAAGGAGGCCTTGGACAAAATTGAGGAGGAGCAAAACAAGAGCAAGAAGAAGGCCAGCAGGCAGC
 TGCTGACACTGGGCATAGCAACCAGGTATCACAGAACTATCCTATTGTCCAAAACATTCAGGGCCAGATGG
 TTCATCAGGCCATCAGCCCCCGGACGCTCAATGCCTGGGTGAAGGTTGTCTGAAGAGAAGGCCTTTTCTCCT
 GAGGTTATCCCCATGTTCTCCGCTTTGAGTGAGGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATAC
 CGTGGGCGGCCATCAGGCCGCCATGCAAATGTTGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACA
 GAGTGATCCCGTCCACGCTGGCCCAATCGCGCCCGGACAGATGCGGGAGCCTCGCGGCTCTGACATTGCC
 GGCACCACCTCTACACTGCAAGAGCAAATCGGATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAAT
 CTATAAACGGTGGATCATTTCTCGGTCTCAATAAAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACA
 TTAGACAGGGACCCAAAGAGCCTTTTAGGGATTACGTCGACCGGTTTTATAAGACCCTGCGAGCAGAGCAG
 GCCTCTCAGGAGGTCAAAAACCTGGATGACGGAGACACTCCTGGTACAGAACGCTAACCCCGACTGCAAAAC
 AATCTTGAAGGCACTAGGCCCGGCTGCCACCCTGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCGGAC
 CCGGACACAAAGCCAGAGTGTTG*ATGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGAC
 TTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACCTCCC
 AAAGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTGGCAGAACTAC
 ACACCAGGGCCAGGGGTCAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGA
 TAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGGATGGATG
 ACCCGGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCACGTGGCCCGAGAGCTG
 CATCCGGAGTACTTCAAGAACTGCTGA [SEQ ID NO: 63]

Figure 11

Plasmid pRix33:



Sequence of insert:

ATGGCCGAGCAGCTGTGGGTACCGTCTACTACGGCGTGCTGTGTGGAAGGAGGCCACGACCACCCTCTT
 CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
 CGGACCCCAACCCCCAGGAGGTGGTGCTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTTCGGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCAGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTTCAGACTCAATTCAGTCAACAGCAGCGTCATGACGCGAGGCCTGCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCTGCCGGATTTCGCGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTCAGCACGGTGCAGTGCACCCATGGCATCCGCCCCGTCTGTGAG
 CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGATCCGGTCGGACAACCTTCATGGACA
 ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCGCAAGATCATCGGCGACATCCGGCA
 GGCCCATTCGAACCTCTCCCGCGCCAGTGAATAACACCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCGAGGG
 CAACAACACAGAGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACGGGCCTG
 CTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCCACC CGGCCAAGCGCCGCTCGTGCAGAGAATGGGTGCCCGAGCTTCGGTACTGTCTGGTGGA
 GAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCCGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGT
 GTGGGCCTCGAGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGTGCGC
 AGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGGTCCTTGATATAACACAGTG
 GCTACCCTCTACTGCGTACACCAGAGGATCGAGATTAAGGATACCAAGGAGGCCTTGGACAAAATTGAGGA
 GGAGCAAAAACAAGAGCAAGAAGAAGGCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCAC
 AGAACTATCCTATTGTCCAAAACATTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCCGGACGCTCAAT
 GCCTGGGTGAAGGTGTGCGAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCCATGTTCTCCGCTTTGAGTGA
 GGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGGCCGCCATGCAAATGT
 TGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCAATCCCGTCCACGCTGGCCCAATCGCG
 CCCGGACAGATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACTCTACACTGCAAGAGCAAATCGG

Figure 11 continued

ATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGGATCATTCTCGGTCTCAATA
AAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAAGAGCCTTTTAGGGAT
TACGTCGACCGGTTTTATAAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAAGTGGATGACGGA
GACACTCCTGGTACAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACCC
TGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCGGACCCGGACACAAAGCCAGAGTGTTGATGGTGGGT
TTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTT
AAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCT
ACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACC
TTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACAC
CAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTG
ACAGCCGCCTAGCATTTTCATCACGTGGCCCGAGAGCTGCATCCGAGTACTTCAAGAACTGCTAA
[SEQ ID NO: 64]

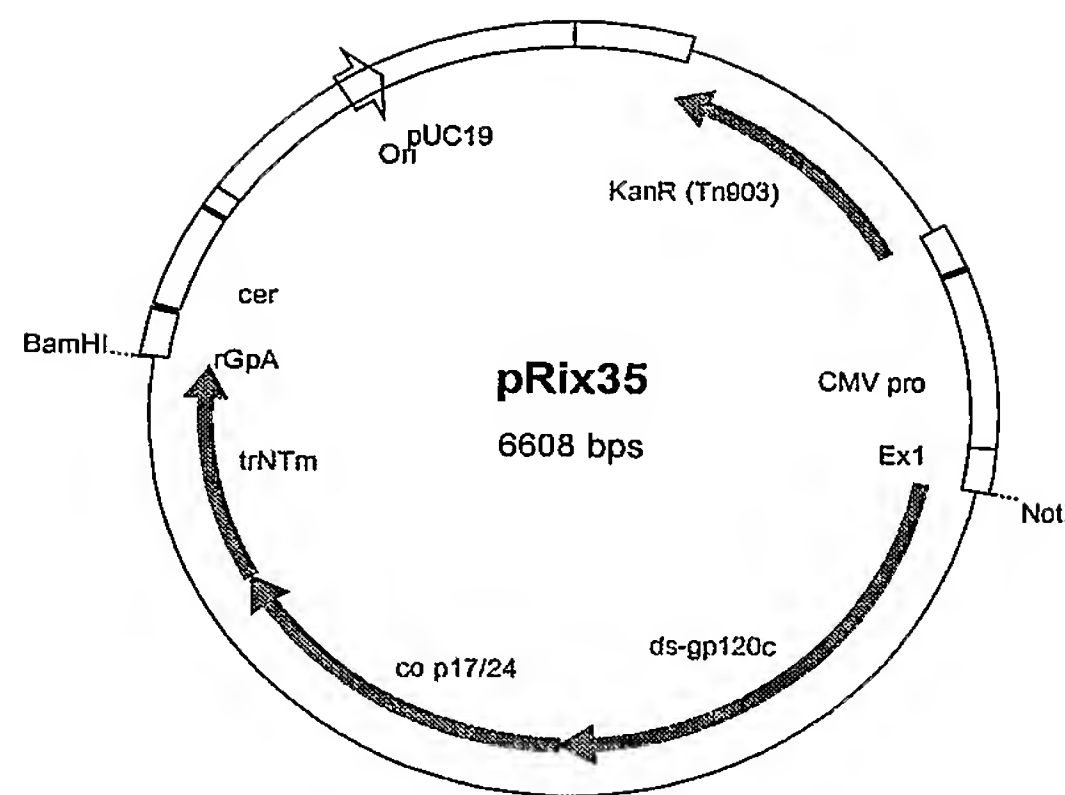
Amino acid sequence of antigen:

MAEQLWVTVYYGVVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEVVLGNVTEYFNMWKNNM
VDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTTSSNGWTGEIRKGEIKNCSFNITTSIRDKV
QKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPPIPIHYCAPAGFAILKCNNKT
FDGKGLCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSDNFMNDTKTIIVQLNESVAINCTRPNNT
RKGIIHIGPGRAFYAARKIIGDIRQAHCNLSRAQWNNTLQKQIVIKLREHFGNKTIKFNQSSGGDPEIVRHSF
NCGGEFFYCDTTQLFNSTWNGTEGNNTGNSTITLPCRIKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
LLTRDGGTEGNGTENETEIFRPGGGDMRDNWRSELYKQVVKVEPLGVAPTRAKRRVVQRMGARASVLSGG
ELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLETSEGCRQILGQLQPSLOTGSEELRSLYNTV
ATLYCVHQRIEIKDTKEALDKIEEEQNKSKKKAQQAADTGHSNQVSQNYPIVQNIQGQMVHQAI SPRTL N
AWVKVVEEKAFSPEVIPMFSALSEGATPQDLNNTMLNTVGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIA
PGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGLNKIVRMYSPTSILDIRQGPKEPFRD
YVDRFYKTLRAEQASQEVKNWMTETLLVQNANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLMVG
FPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHTQGYFPDWQNYTPGPGVRYPLT
FGWCYKLVPEPDKVEEANKGENTSLHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNC

[SEQ ID NO: 65]

Figure 12

Plasmid pRix35:



Sequence of insert

ATGGCCGAGCAGCTGTGGGTCACCGTCTACTACGGCGTGCCTGTGTGGAAGGAGGCCACGACCACCCTCTT
 CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
 CGGACCCCAACCCCAAGGAGGTGGTGTCTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTGGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTGAGACTCATTCAGTCAACAGCAGCGTCATGACGCGAGGCCCTGCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCTGCCGGATTGCGGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCCGCCCCGTGCTGAG
 CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGATCCGGTCCGACAACCTTCATGGACA
 ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCCGCAAGATCATCGGCGACATCCGGCA
 GGCCCATTTGCAACCTCTCCCGCGCCAGTGGAATAACACCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCGAGGG
 CAACAACACAGAGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTG
 CTGCTCACCAGAGACGGGGGACCCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCCACC CGGGCCAAGCGCCGCGTCGTGCAGAGAATGGGTGCCCGAGCTTCGGTACTGTCTGGTGA
 GAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCCGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGT
 GTGGGCCTCGAGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGTGCGC
 AGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGGTCCTTGTATAACACAGTG
 GCTACCCTCTACTGCGTACACCAGAGGATCGAGATTAAGGATACCAAGGAGGCCTTGGACAAAATTGAGGA
 GGAGCAAAAACAAGAGCAAGAAGAAGGCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCAC
 AGAACTATCCTATTGTCCAAAACATTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCGGACGCTCAAT
 GCCTGGGTGAAGGTTGTGGAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCCATGTTCTCCGCTTTGAGTGA
 GGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGGCCGCCATGCAAAATGT
 TGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCG
 CCGGACAGATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACTCTACACTGCAAGAGCAAATCGG

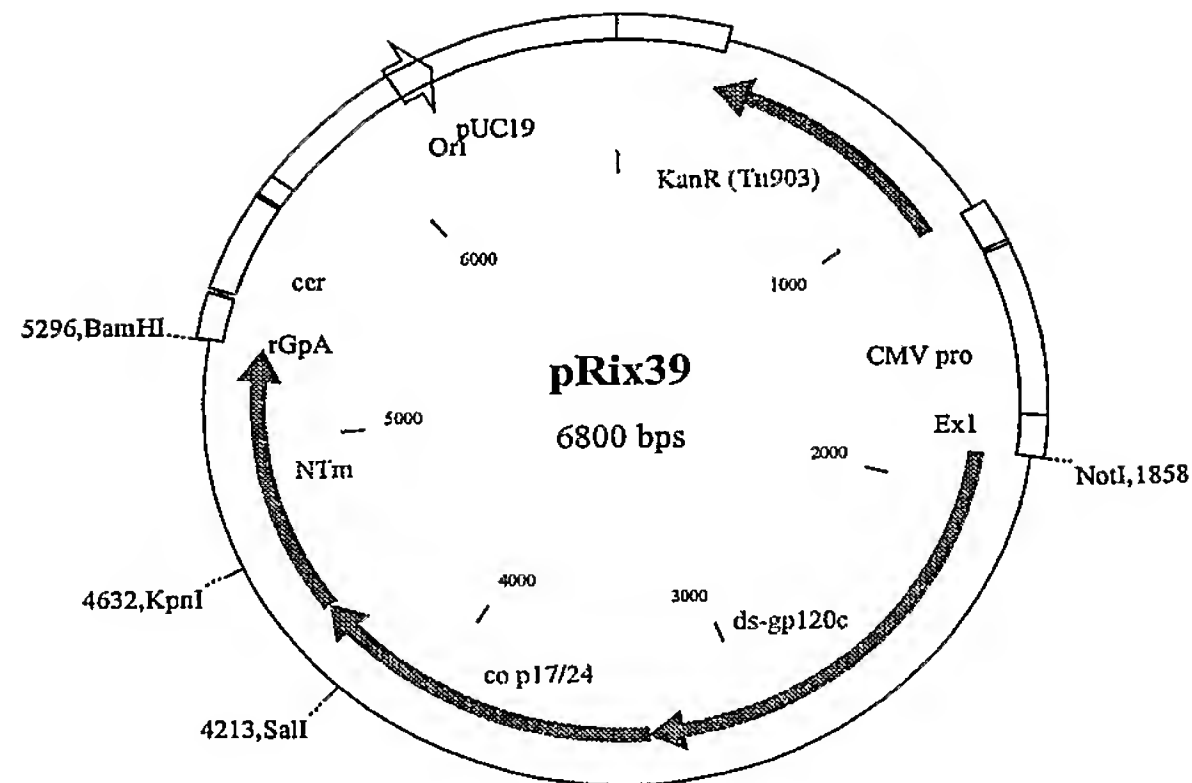
Figure 12 continued

ATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGGATCATTCTCGGTCTCAATA
 AAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAAGAGCCTTTTAGGGAT
 TACGTCGACCGGTTTTATAAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAACCTGGATGACGGA
 GACACTCCTGGTACAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACCC
 TGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCGGACCCGGACACAAAGCCAGAGTGTTGATGGTGGGT
 TTTCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTT
 AAAAGAAAAGGGGGGACTGGAAGGGCTAATTCCTCCAACGAAGACAAGATATCCTTGATCTGTGGATCT
 ACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACC
 TTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACAC
 CAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGAGGTTTG
 ACAGCCGCCTAGCATTTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAG
 CCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACCTGCTTGTACCAATTGCTA
 TTGTAAAAAGTGTTGCTTTTCATTGCCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCTATGGCAGGA
 AGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCC
 ACCTCCCAATCCAAAGGGGAGCCGACAGGCCCGAAGGAATAA [SEQ ID NO: 66]

Amino acid sequence of antigen:

MAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVVLGNVTEYFNMWKNM
 VDQMHEIDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTTSNGWTGEIRKGEIKNCSFNITTSIRDKV
 QKEYALFYNLDVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNNKT
 FDGKGLCTNVSTVQCTHGIRPVVSTQLLLNGSLAESEVVIRSDNFMDNTKTIIVQLNESVAINCTRPNNNT
 RKGIIHIGPGRFYAARKIIGDIRQAHCNLSRAQWNNTLKQIVIKLREHFGNKTIKFNQSSGGDPEIVRHSF
 NCGGEFFYCDTTQLFNSTWNGTEGNNTGNSTITLPCRIKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
 LLTRDGGTEGNGTENETEIFRPGGDMRDNRSELYKYKVVEPLGVAPTRAKRRVVQRMGARASVLSGG
 ELDRWEKIRLRPGGKKKYKLKHIIVWASRELERFAVNPGLLETSEGCRQILGQLQPSLQTGSEELRSLYNTV
 ATLYCVHQRIEIKDTKEALDKIEEEQNKSKKKAQQAADTGHSNQVSQNYPIVQNIQGMVHQAI SPRTL N
 AWWKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIA
 PGQMPREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGLNKIVRMYSPTSILDIRQGPKEPFRD
 YVDRFYKTLRAEQASQEVKNWMTETLLVQANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLMVG
 FPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLDWIYHTQGYFPDWQNYTPGPGVRYPLT
 FGWCYKLVPEPDKVEEANKGENTSLLHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSE
 PVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQP
 TSQSKGEPTGPKE [SEQ ID NO: 67]

Figure 13



Sequence of insert:

ATGGCCGAGCAGCTGTGGGTCACCGTCTACTACGGCGTGCTGTGTGGAAGGAGGCCACGACCACCCTCTT
 CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
 CGGACCCCAACCCCAAGGAGGTGGTGTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTGGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTACAGACTCATTTCACTGCAACAGCAGCGTCATGACGCAGGCCTGCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCTGCCGATTTCGCGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCCGCCCCGTCGTGAG
 CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGTATCCGGTTCGGACAACCTTCATGGACA
 ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACCTGTACCCGGCCTAACAACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCGCAAGATCATCGGCGACATCCGGCA
 GGCCCATTGCAACCTCTCCCGCGCCCAGTGGAATAACACCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCGAGGG
 CAACAACACAGAGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTG
 CTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCCACCCGGGCCAAGCGCCGCGTGTGTCAGAGAAATGGGTGCCGAGCTTCGGTACTGTCTGGTGA
 GAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGT
 GTGGGCCTCGAGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGTCGCC
 AGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGGTCCTTGTATAACACAGTG
 GCTACCTCTACTGCGTACACCAGAGGATCGAGATTAAGGATACCAAGGAGGCCTTGGACAAAATTGAGGA
 GGAGCAAAAACAAGAGCAAGAAGAAGGCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCAC
 AGAACTATCCTATTGTCCAAAACATTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCGGACGCTCAAT
 GCCTGGGTGAAGTTGTGCAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCATGTTCTCCGCTTTGAGTGA
 GGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGGCCGCCATGCAAATGT
 TGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCG
 CCCGGACAGATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACTCTACACTGCAAGAGCAAAATCGG
 ATGGATGACCAACAATCCTCCCATCCAGTTGGAGAAATCTATAACGGTGGATCATTTCTCGGTCTCAATA
 AAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAAGAGCCTTTTAGGGAT

Figure 13 continued

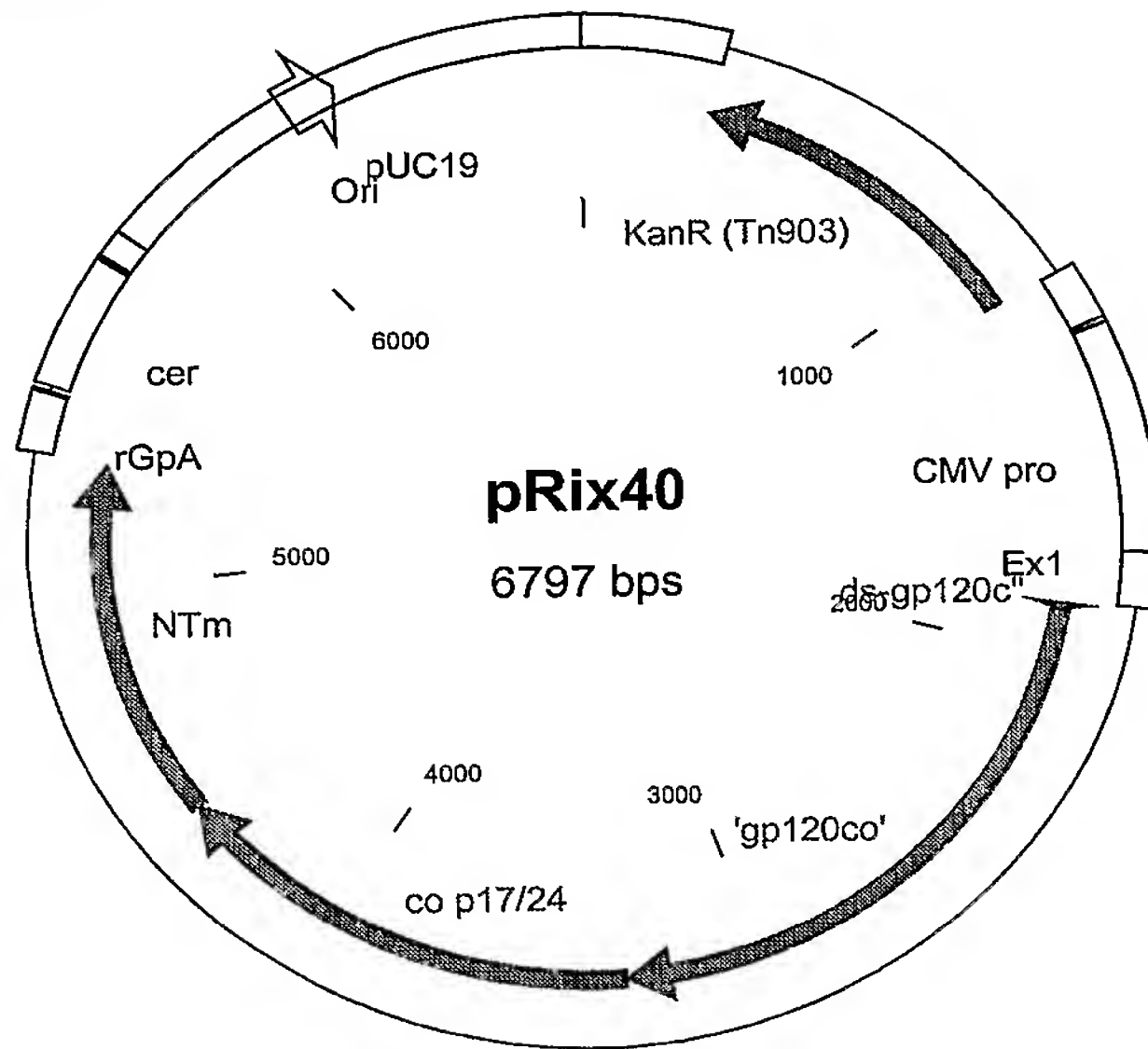
TACGTCGACCGGTTTTATAAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAAGTGGATGACGGA
 GACACTCCTGGTACAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCCTAGGCCCGGCTGCCACCC
 TGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCCGGACCCGGACACAAAGCCAGAGTGTTGATGGGTGGC
 AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCAGCAGC
 AGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACAAGTAGCAATACAGCAGCTA
 CCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTA
 CCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGGGGACTGGA
 AGGGCTAATTCCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCC
 CTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACCTTTGGATGGTGCTACAAGCTA
 GTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAG
 CCTGCATGGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATC
 ACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTAGAG
 CCCTGGAAGCATCCAGGAAGTCAGCCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTGCTTTCA
 TTGCCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAA
 GACCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCAAGGGGAG
 CCGACAGGCCCGAAGGAATAA [SEQ ID NO: 68]

Amino acid sequence of antigen:

MAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVVLGNVTEYFNMWKNM
 VDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTTNSNGWTGEIRKGEIKNCSFNITTSIRDKV
 QKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNKNT
 FDGKGLCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSDNFMDNTKTIIVQLNESVAINCTRPNNNT
 RKGIIHIGPGRAFYAARKIIGDIRQAHCNLSRAQWNNTLKQIVIKLREHFGNKTIKFNQSSGGDPEIVRHSF
 NCGGEFFYCDTTQLFNSTWNGTEGNNTGNSTITLPCRICKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
 LLTRDGGTEGNGTENETEIFRPGGDMRDNRSELYKYKVKVEPLGVAPTRAKRRVVQRMGARASVLSGG
 ELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLEETSEGCRQILGQLQPSLQTGSEELRSLYNTV
 ATLYCVHQRIEIKDTKEALDKIEEEQNKSKKKAQQAADTGHSNQVSQNYPIVQNIQGQMVHQAI SPRTL N
 AWWKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIA
 PGQMREPRGSDIAGTTSTLQEQIGWMTNPNPPIPVGEIYKRWIILGLNKIVRMYSPTSILDIRQGPKEPFRD
 YVDRFYKTLRAEQASQEVKNWMTETLLVQNANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLMGG
 KWSKSSVVGWPTVRRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQEEEEVGFVTPQV
 PLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLDWIYHTQGYFPDWQNYTPGPGVRYPLTFGWCYKL
 VPVEPDKVEEANKGENTSLLHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLE
 PWKHPGSQPKTACTNCYCKKCCFHCQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSKGE
 PTGPKE [SEQ ID NO: 69]

Figure 14

pRix40

**DNA sequence of insert**

ATGGCCGAGCAGCTGTGGGTACCGTCTACTACGGCGTGCCTGTGTGGAAGGAGGCCACGACCACCTCTT
 CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
 CGGACCCCAACCCCAAGGAGGTGGTGTCTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTTCGGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTTCAGACTCATTCACTGCAACAGCAGCGTCATGACGCGAGGCCTGCCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCTGCCGGATTTCGCGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCCGCCCCGTCTGTGAG
 CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGTATCCGGTCGGACAACCTTCATGGACA
 ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCCGCAAGATCATCGGCGACATCCGGCA
 GGCCCATTTGCAACCTCTCCCGCGCCAGTGGAATAACACCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCGAGGG
 CAACAACACAGAGGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTG
 CTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCCACC CGGGCCAAGCGCCGCGTCTGTCAGAGAATGGGTGCCCGAGCTTCGGTACTGTCTGGTGGA
 GAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGT
 GTGGGCCTCGAGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGTCGCC
 AGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGGTCCTTGTATAACACAGTG
 GCTACCTCTACTGCGTACACCAGAGGATCGAGATTAAGGATACCAAGGAGGCCTTGGACAAAATTGAGGA
 GGAGCAAAACAAGAGCAAGAAGAAGGCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCAC
 AGAACTATCCTATTGTCCAAAACATTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCCGGACGCTCAAT
 GCCTGGGTGAAGGTTGTCTGAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCATGTTCTCCGCTTTGAGTGA
 GGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGGCCCGCCATGCAAATGT
 TGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCG

Figure 14 continued

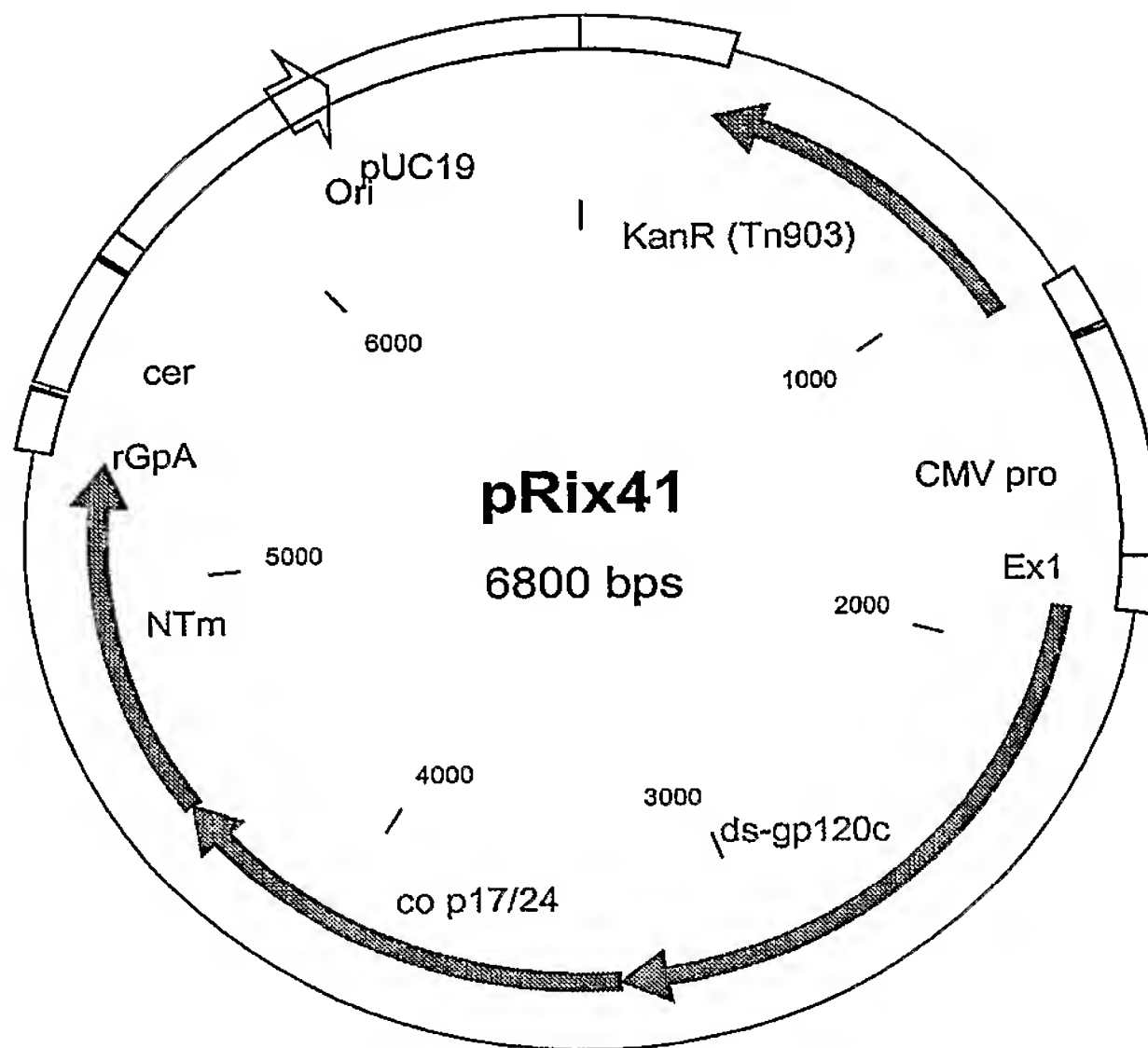
CCCGGACAGATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAATCGG
ATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGGATCATTCTCGGTCTCAATA
AAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAAGAGCCTTTTAGGGAT
TACGTGACCGGTTTTATAAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAAGTGGATGACGGA
GACACTCCTGGTACAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACCC
TGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCGGACCCGGACACAAAGCCAGAGTGTTGATGGGCAAG
TGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCAGCAGCAGA
TGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCA
ATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCT
TTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGGGGACTGGAAGG
GCTAATTCACCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTG
ATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTA
CCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCT
GCATGGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTTGACAGCCGCCTAGCATTTCATCACG
TGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTAGAGCCC
TGGAAGCATCCAGGAAGTCAGCCTAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTTCATTG
CCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGAC
CTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCAAGGGGAGCCG
ACAGGCCCGAAGGAATAA [SEQ ID NO: 70]

Aminoacid sequence of insert

MAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVVLGNVTEYFNMWKNM
VDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTNSTTTTNSNGWTGEIRKGEIKNCSFNITTSIRDKV
QKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNNKT
FDGKGLCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEVVIRSDNFMNTKTIIVQLNESVAINCTRPNNNT
RKGIHIGPGRAFYAARKIIGDIRQAHCNLSRAQWNNTLQKQIVIKLREHFGNKTIFKNQSSGGDPEIVRHSF
NCGGEFFYCDTTQLFNSTWNGTEGNNTGNSTITLPCRKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
LLTRDGGTEGNGTENETEIFRPGGGDMRDNRSELYKYKVVKVEPLGVAPTRAKRRVVQRMGARASVLSGG
ELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLETSEGCRQILGQLQPSLOTGSEELRSLYNTV
ATLYCVHQRIEIKDTKEALDKIEEQNKSKKKAQQAADTGHSNQVSQNYPIVQNIQGQMVHQAI SPRTL N
AWVKVVEEKAFSPEVIPMFSALSEGATPQDLNNTMLNTVGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIA
PGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGLNKIVRMYSPTSILDIRQGPKEPFRD
YVDRFYKTLRAEQASQEVKNWMTETLLVQANANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLMGK
WSKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQEEEEVGFPVTPQVP
LRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHTQGYFPDWQNYTPGPGVRYPLTFGW CYKLV
PVEPDKVEEANKGENTSLLHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEP
WKHPGSQPKTACTNCYCKKCCFHCQVCFITAAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSKGEP
TGPKE [SEQ ID NO: 71]

Figure 15

pRix41

**DNA sequence of insert**

ATGGCCGAGCAGCTGTGGGTCACCGTCTACTACGGCGTGCCTGTGTGGAAGGAGGCCACGACCACCTCTT
 CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
 CGGACCCCAACCCCAAGGAGGTGGTGGTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTTCGGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTTCAGACTCATTCACTGCAACAGCAGCGTCATGACGCGAGGCCTGCCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCTGCCGGATTTCGCGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCCGCCCCGTGCTGAG
 CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGATCCGGTCGGACAACCTTCATGGACA
 ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCGCAAGATCATCGGCGACATCCGGCA
 GGCCCATTTGCAACCTCTCCCGCGCCCAAGTGAATAAACCCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCGAGGG
 CAACAACACAGAGGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTG
 CTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCCACC CGGGCCAAGCGCCGCGTCTGTCAGAGAATGGGTGCCCGAGCTTCGGTACTGTCTGGTGGGA
 GAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGT
 GTGGGCCTCGAGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGTCGCC
 AGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGGTCCTTGTATAACACAGTG
 GCTACCTCTACTGCGTACACCAGAGGATCGAGATTAAGGATACCAAGGAGGCCTTGGACAAAATTGAGGA
 GGAGCAAAACAAGAGCAAGAAGAAGGCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCAC
 AGAACTATCCTATTGTCCAAAACATTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCCGGACGCTCAAT
 GCCTGGGTGAAGGTTGTGCAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCCATGTTCTCCGCTTTGAGTGA
 GGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGGCCGCCATGCAAAATGT

Figure 15 continued

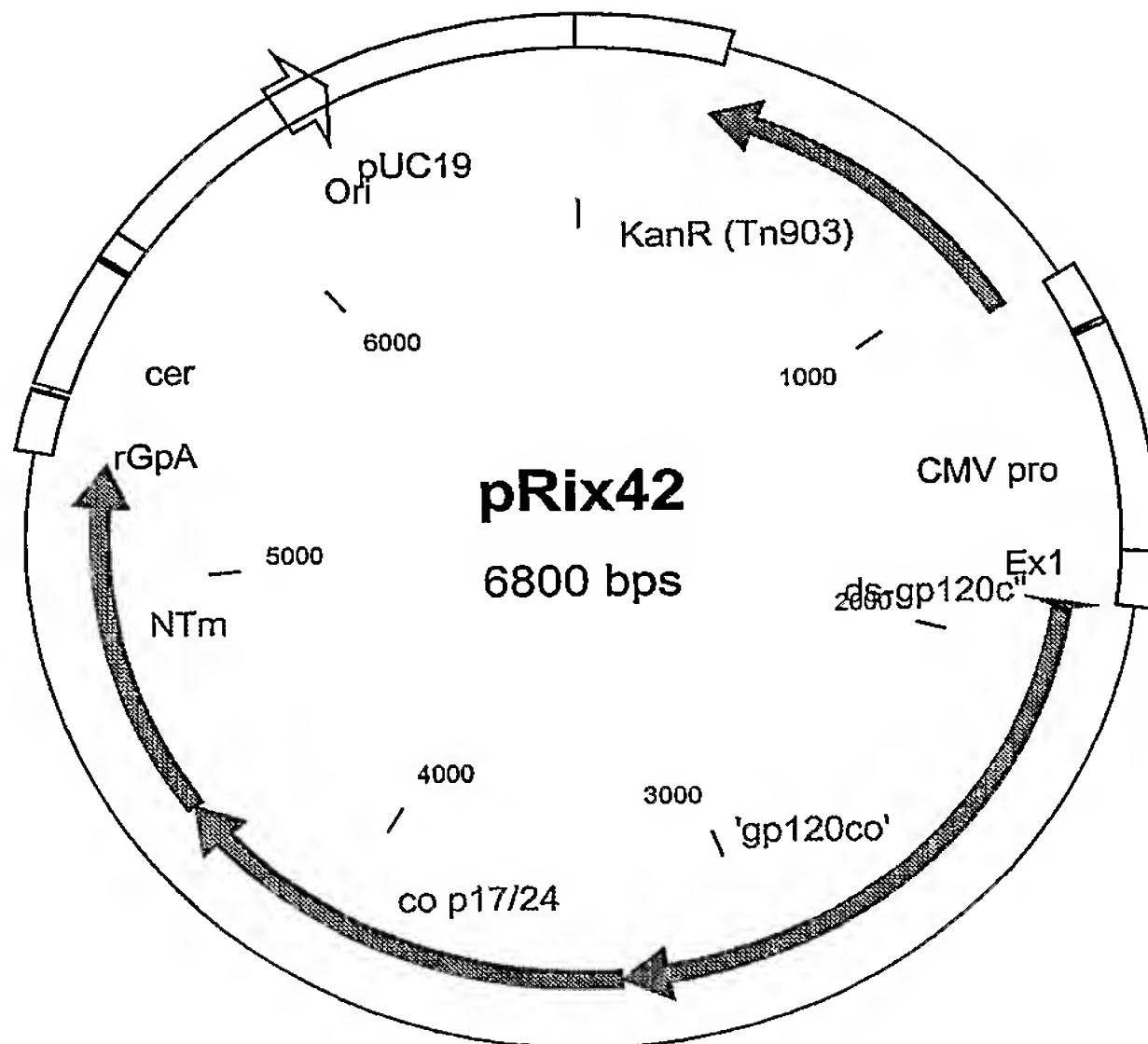
TGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCG
 CCCGGACAGATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAATCGG
 ATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGGATCATTCTCGGTCTCAATA
 AAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAAGAGCCTTTTAGGGAT
 TACGTCGACCGGTTTTATAAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAAGTGGATGACGGA
 GACACTCCTGGTACAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACCC
 TGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCGGACCCGGACACAAAGCCAGAGTGTGATGGGTGGC
 AAGTGGTCAAAAAGTAGTGTGGTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCAGCAGC
 AGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACAAGTAGCAATACAGCAGCTA
 CCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTA
 CCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGGGGACTGGA
 AGGGCTAATCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCC
 CTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACCTTTGGATGGTGCTACAAGCTA
 GTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCGCCTTACACCCTGTGAG
 CCTGCATGGAATGGATGACCCTGAGAGAGAAGTGTAGAGTGGAGGTTTGACAGCCCGCCTAGCATTTTCATC
 ACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTAGAG
 CCCTGGAAGCATCCAGGAAGTCAGCCTAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTGCTTTCA
 TTGCCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAA
 GACCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCAAAGGGGAG
 CCGACAGGCCCGAAGGAATAA [SEQ ID NO: 72]

Aminoacid sequence of insert

MAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVVLGNVTEYFNMWKNM
 VDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTNSTTTTSSNGWTGEIRKGEIKNCSFNITTSIRDKV
 QKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPPIPIHYCAPAGFAILKCNNKT
 FDGKGLCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEVVIRSDNFMDNTKTIIVQLNESVAINCTRPNNT
 RKGIIHIGPGRAFYAARKIIGDIRQAHCNLSRAQWNNTLQKQIVIKLREHFGNKTIFNQSSGGDPEIVRHSF
 NCGGEFFYCDTTQLFNSTWNGTEGNNTGNSTITLPCRKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
 LLTRDGGTEGNGTENETEIFRPGGGDMRDNRSELYKYKVVKVEPLGVAPTRAKRRVVQRMGARASVLSGG
 ELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLETSEGCRQILGQLQPSLQTGSEELRSLYNTV
 ATLYCVHQRIEIKDTKEALDKIEEQNKSKKKAQQAADTGHSNQVSQNYPIVQNIQGQMVHQAI SPRTLN
 AWWKVVEEKAFSPEVIMFSALEGATPQDLNMTLNTVGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIA
 PGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGLNKIVRMYSPTSILDIRQGPKEPFRD
 YVDRFYKTLRAEQASQEVKNWMTETLLVQANANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLMGG
 KWSKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSNTAATNAACAWLEAQEEEEVGFVTPQV
 PLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWYHTQGYFPDWQNYTPGPGVRYPLTFGWCYKL
 VPVEPKVEEANKGENTSALHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLE
 PWKHPGSQPKTACTNCYCKKCCFHCQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSKGE
 PTGPKE [SEQ ID NO: 73]

Figure 16

pRix42

**DNA sequence of insert**

ATGGCCGAGCAGCTGTGGGTCACCGTCTACTACGGCGTGCCTGTGTGGAAGGAGGCCACGACCACCTCTT
 CTGCGCGAGCGACGCCAAGGCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
 CGGACCCCAACCCCAAGGAGGTGGTGTCTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTTCGGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTTCAGACTCATTCACTGCAACAGCAGCGTCATGACGCGAGGCTGCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCTGCCGGATTTCGCGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTCAGCACGGTGCAGTGCACCCATGGCATCCGCCCCGTCTGTGAG
 CACCCAGCTGCTGTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGTATCCGGTCCGACAACTTCATGGACA
 ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCGCAAGATCATCGGCGACATCCGGCA
 GGCCATTGCAACCTCTCCCGCGCCAGTGGAAATAACACCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCAGGGG
 CAACAACACAGAGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTG
 CTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCCACC CGGGCCAAGCGCCGCTCGTGACAGAGAATGGGTGCCCGAGCTTCGGTACTGTCTGGTGGG
 GAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCCGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGT
 GTGGGCCTCGAGGGAGCTTGAACGGTTTGGCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGTGCGC
 AGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGGTCCTTGTATAACACAGTG
 GCTACCCTCTACTGCGTACACCAGAGGATCGAGATTAAGGATACCAAGGAGGCCTTGGACAAAATTGAGGA
 GGAGCAAAACAAGAGCAAGAAGAAGGCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCAC
 AGAACTATCCTATTGTCCAAAACATTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCGGACGCTCAAT
 GCCTGGGTGAAGGTTGTGCAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCCATGTTCTCCGCTTTGAGTGA
 GGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGGCCGCCATGCAAAATGT

Figure 16 continued

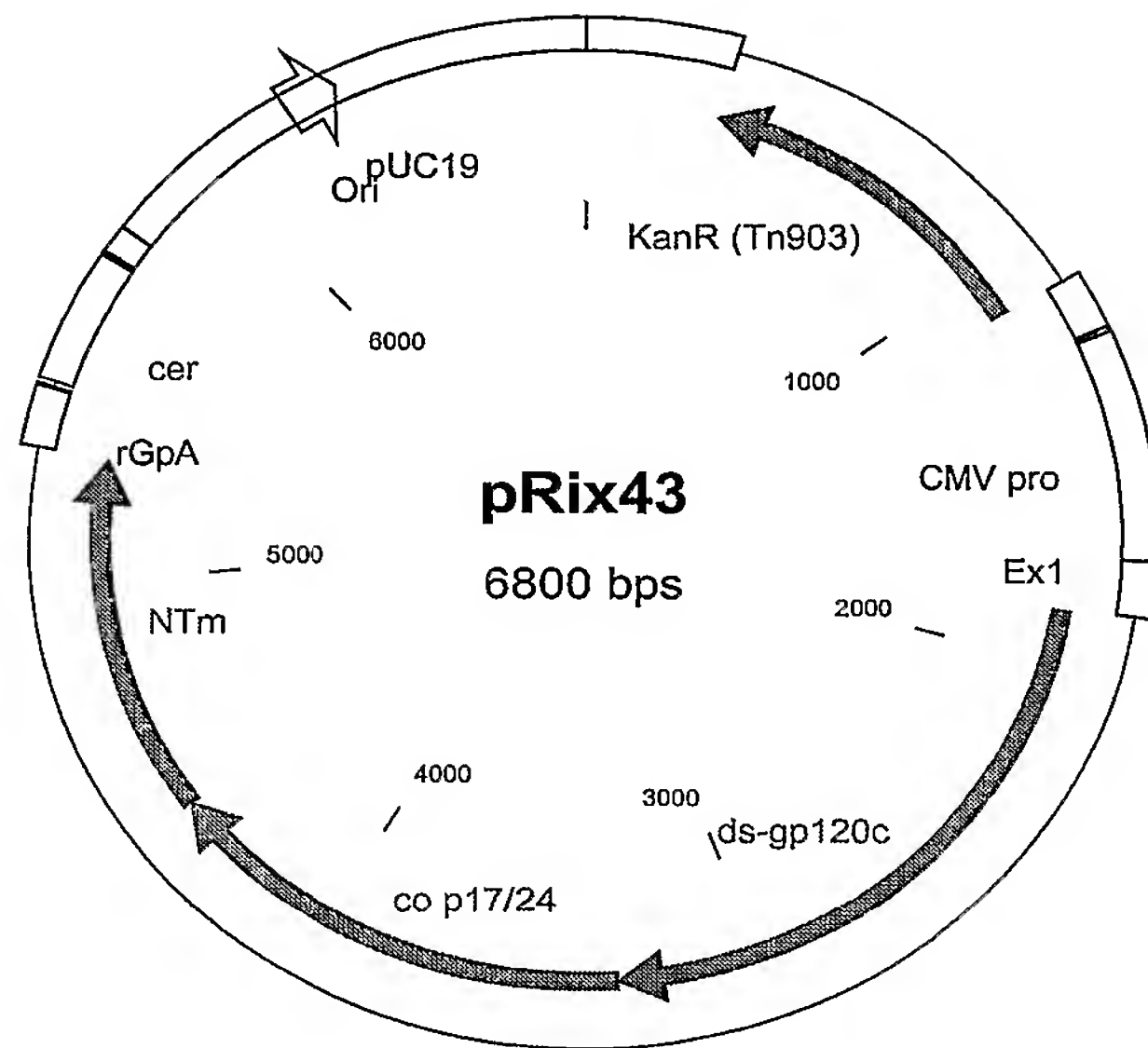
TGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCG
 CCCGGACAGATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAATCGG
 ATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGGATCATTCTCGGTCTCAATA
 AAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAAGAGCCTTTTAGGGAT
 TACGTCGACCGGTTTTATAAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAACCTGGATGACGGA
 GACACTCCTGGTACAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACCC
 TGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCGGACCCGGACACAAAGCCAGAGTGTGATGGGTGGC
 AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCAGCAGC
 AGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACAAGTAGCAATACAGCAGCTA
 CCAATGCTGCTTGTGCCTGGCTAGAAGCACAAAGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTA
 CCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGA
 AGGGCTAATTCACCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCC
 CTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTTGGATGGTGCTACAAGCTA
 GTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGGCACACCCTGTGAG
 CCTGCATGGAATGGATGACCCTGAGAGAGAAGTGTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATC
 ACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTAGAG
 CCCTGGAAGCATCCAGGAAGTCAGCCTAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTGCTTTCA
 TTGCCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAA
 GACCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCAAAGGGGAG
 CCGACAGGCCCGAAGGAATAA [SEQ ID NO: 74]

Aminoacid sequence of insert

MAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVVLGNVTEYFNMWKNM
 VDMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTTNSNGWTGEIRKGEIKNCSEFNITTSIRDKV
 QKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNNKT
 FDGKGLCTNVSTVQCTHGIRPVVSTQLLLNGSLAEFEVIRSDNFMNTKTIIVQLNESVAINCTRPNNT
 RKGIIHIGPGRFYAARKIIGDIRQAHCNLSRAQWNNTLKQIVIKLREHFGNKTIKFNQSSGGDPEIVRHSF
 NCGGEFFYCDTTQLFNSTWNGTEGNNTGNSTITLPCRICKIINMWQEVGKAMYAPPIGGQIRCSSNITGL
 LLTRDGGTEGNGTENETEIFRPGGGDMRDNRSELYKYKVVKVEPLGVAPTRAKRRVVQRMGARASVLSGG
 ELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLETSEGCRQILGQLQPSLQTGSEELRSLYNTV
 ATLYCVHQRIEIKDTKEALDKIEEQNKSKKKAQQAADTGHSNQVSQNYPIVQNIQGQMVHQAI SPRTLN
 AWWKVVEEKAFSPEVIPMFSALSEGATPQDLNMTLNTVGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIA
 PGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGLNKIVRMYSPTSILDIRQGPKEPFRD
 YVDRFYKTLRAEQASQEVKNWMTETLLVQANANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLMGG
 KWSKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQEEEEVGFPVTPQV
 PLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWIIYHTQGYFPDWQNYTPGPGVRYPLTFGWCYKL
 VPVEPDKVEEANKGENTSLAHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLE
 PWKHPGSQPKTACTNCYCKKCCFHCQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSKGE
 PTGPKE [SEQ ID NO: 75]

Figure 17

pRix43



DNA sequence of insert

ATGGCCGAGCAGCTGTGGGTCACCGTCTACTACGGCGTGCTGTGTGGAAGGAGGCCACGACCACCCTCTT
 CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCCTA
 CGGACCCCAACCCCAAGGAGGTGGTGCTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTTCGGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTTCAGACTCATTCACTGCAACAGCAGCGTCATGACGCGAGGCCTGCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCTGCCGGATTCGCGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCCGCCCCGTCGTGAG
 CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGATCCGGTCGGACAACCTTCATGGACA
 ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCGCAAGATCATCGGCGACATCCGGCA
 GGCCCATTGCAACCTCTCCCGCGCCAGTGGAATAACACCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCGAGGG
 CAACAACACAGAGGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTG
 CTGCTCACCAAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCCACCCGGGCCAAGCGCCGCGTCTGTCAGAGAATGGGTGCCCGAGCTTCGGTACTGTCTGGTGA
 GAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGT
 GTGGGCCTCGAGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGTGCC
 AGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGGTCCTTGTATAACACAGTG
 GCTACCCTCTACTGCGTACACCAAGAGGATCGAGATTAAGGATACCAAGGAGGCCTTGGACAAAATTGAGGA
 GGAGCAAAACAAGAGCAAGAAGAAGGCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCAC
 AGAACTATCCTATTGTCCAAAACATTTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCCGGACGCTCAAT
 GCCTGGGTGAAGGTTGTGCAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCCATGTTCTCCGCTTTGAGTGA
 GGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGGCCGCCATGCAAATGT

Figure 17 continued

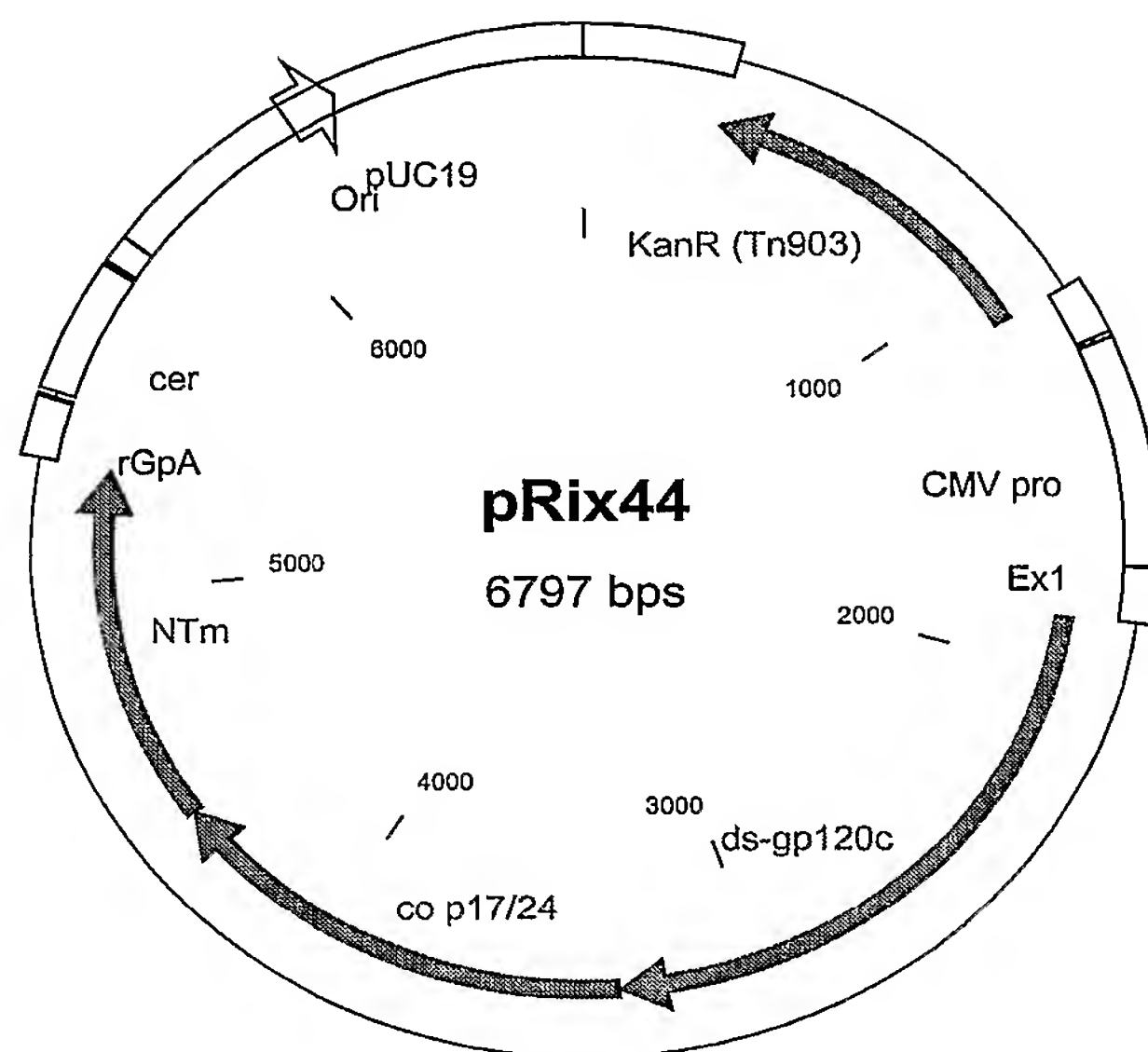
TGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCG
 CCCGGACAGATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAATCGG
 ATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGGATCATTCTCGGTCTCAATA
 AAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAAGAGCCTTTTAGGGAT
 TACGTGACCGGTTTTATAAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAACCTGGATGACGGA
 GACTCCTGGTACAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACCC
 TGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCGGACCCGGACACAAAGCCAGAGTGTTGATGGGTGGC
 AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCAGCAGC
 AGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACAAGTAGCAATACAGCAGCTA
 CCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTA
 CCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGGGGACTGGA
 AGGGCTAATTCCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCC
 CTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTTGGATGGTGCTACAAGCTA
 GTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCGCCGCACACCCTGTGAG
 CCTGCATGGAATGGATGACCTGAGAGAGAAGTGTTAGAGTGAGGTTTTGACAGCCGCCTAGCATTTCATC
 ACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTAGAG
 CCCTGGAAGCATCCAGGAAGTCAGCCTAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCA
 TTGCCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAA
 GACCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCAAAGGGGAG
 CCGACAGGCCCGAAGGAATAA [SEQ ID NO: 76]

Aminoacid sequence of insert

MAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEVVLGNVTEYFNMWKNM
 VDQMHEIDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTTSSNGWTGEIRKGEIKNCSFNITTSIRDKV
 QKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNKNT
 FDGKGLCTNVSTVQCTHGIRPVVSTQLLNLGSLAESEVVIRSDNFMDNTKTIIVQLNESVAINCTRPNNT
 RKGIIHIGPGRFYAARKIIGDIRQAHCNLSRAQWNNTLKKQIVIKLREHFGNKTIKFNQSSGGDPEIVRHSF
 NCGGEFFYCDTTQLFNSTWNGTEGNNTTEGNSTITLPCRIKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
 LLTRDGGTEGNGTENETEIFRPGGGDMRDNRSELYKYKVVKVEPLGVAPTRAKRRVVQRMGARASVLSGG
 ELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLETSEGCRQILGQLQPSLQTGSEELRSLYNTV
 ATLYCVHQRIEIKDTKEALDKIEEEQNKSKKKAQQAADTGHSNQVSQNYPIVQNIQGQMVHQAI SPRTL
 AWWKVVEEKAFSPFVPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIA
 PGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGLNKIVRMYSPITSILDIRQGPKEPFRD
 YVDRFYKTLRAEQASQEVKNWMTETLLVQNANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLMGG
 KWSKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSNTAATNAACAWLEAQEEEEVGFPVTPQV
 PLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWYHTQGYFPDWQNYTPGPGVRYPLTFGWICYKL
 VPVEPDKVEEANKGENTSAAHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLE
 PWKHPGSQPKTACTNCYCKKCCFHCQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSKGE
 PTGPKE [SEQ ID NO: 77]

Figure 18

pRix44



DNA sequence of insert

ATGGCCGAGCAGCTGTGGGTACCGTCTACTACGGCGTGCCTGTGTGGAAGGAGGCCACGACCACCCTCTT
 CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
 CGGACCCCAACCCCAAGGAGGTGGTGTCTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTTCGGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTTCAGACTCATTCACTGCAACAGCAGCGTCATGACGCAGGCCTGCCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCTGCCGGATTCGCGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCCGCCCCGTCTGTGAG
 CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGATCCGGTCGGACAACCTTCATGGACA
 ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCCGCAAGATCATCGGCGACATCCGGCA
 GGCCCATTTGCAACCTCTCCCGCGCCCAAGTGAATAACACCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCGAGGG
 CAACAACACAGAGGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTG
 CTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCCACCCGGGCCAAGCGCCGCGTCTGTCAGAGAATGGGTGCCCGAGCTTCGGTACTGTCTGGTGGGA
 GAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGT
 GTGGGCCTCGAGGGAGCTTGAACGGTTTGGCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGTCGCC
 AGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGGTCCTTGTATAACACAGTG
 GCTACCTCTACTGCGTACACCAGAGGATCGAGATTAAGGATACCAAGGAGGCCTTGGACAAAATTGAGGA
 GGAGCAAAACAAGAGCAAGAAGAAGGCCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCAC
 AGAACTATCCTATTGTCCAAAACATTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCCGGACGCTCAAT
 GCCTGGGTGAAGGTTGTCTGAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCCATGTTCTCCGCTTTGAGTGA
 GGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGGCCGCCATGCAAATGT

Figure 18 continued

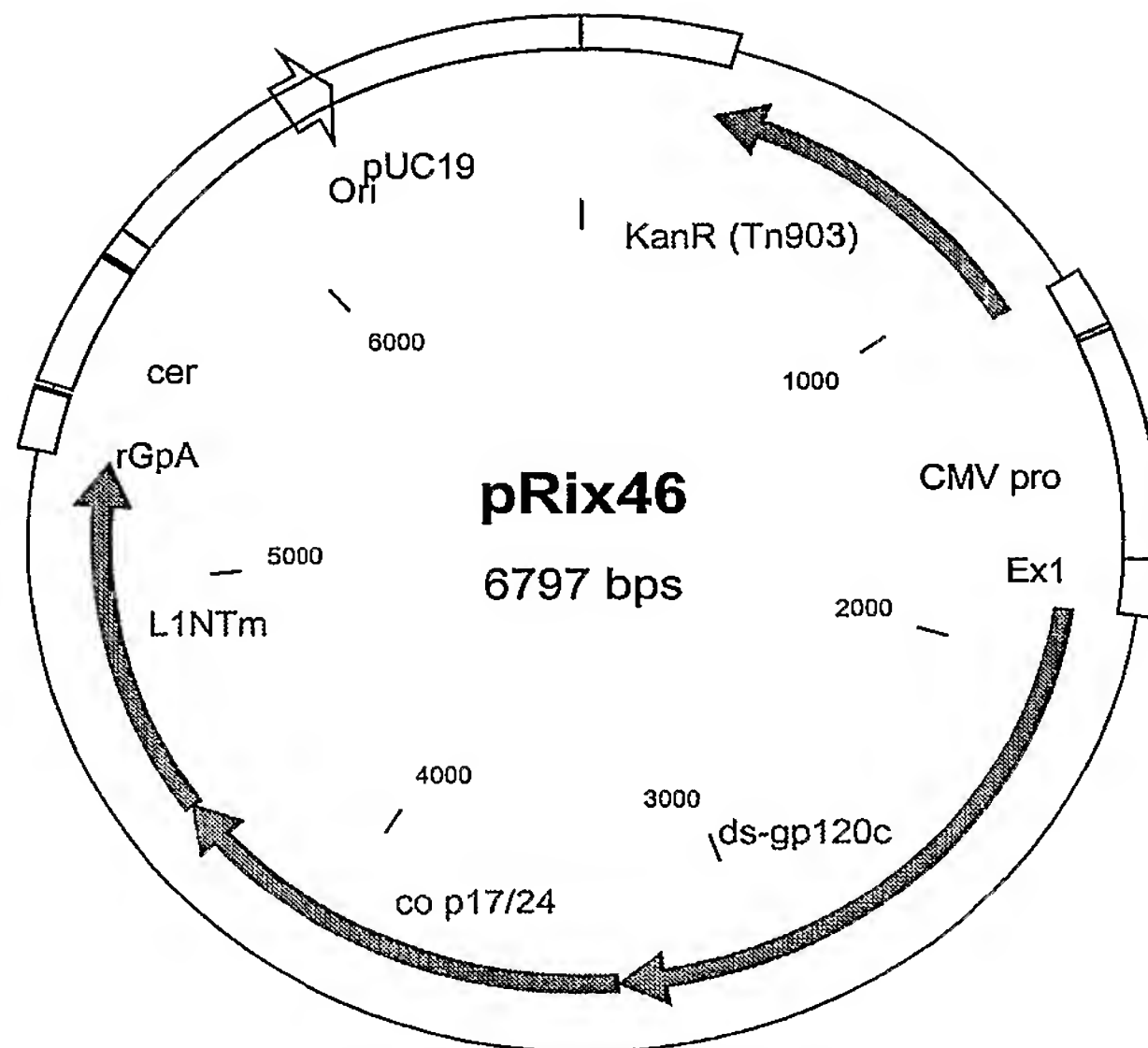
TGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCG
CCCGGACAGATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAATCGG
ATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGGATCATTCTCGGTCTCAATA
AAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAAGAGCCTTTTAGGGAT
TACGTGACCGGTTTTTATAAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAACCTGGATGACGGA
GACACTCCTGGTACAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACCC
TGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCGGACCCGGACACAAAGCCAGAGTGTTGATGGGCAAG
TGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCAGCAGCAGA
TGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCA
ATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCT
TTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGGGGACTGGAAGG
GCTAATTCACCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTG
ATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTA
CCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCGCCGCACACCCTGTGAGCCT
GCATGGAATGGATGACCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCACG
TGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTAGAGCCC
TGGAAGCATCCAGGAAGTCAGCCTAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCATTG
CCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGAC
CTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCAAAGGGGAGCCG
ACAGGCCCCGAAGGAATAA [SEQ ID NO: 78]

Aminoacid sequence of insert

MAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVVLGNVTEYFNMWKNM
VDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTTNSNGWTGEIRKGEIKNCSFNITTSIRDKV
QKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNKKT
FDGKGLCTNVSTVQCTHGIRPVVSTQLLNGSLAEDEVIRSDNFMDNTKTIIVQLNESVAINCTRPNNT
RKGIIHIGPGRAFYAARKIIGDIRQAHCNLSRAQWNNTLKQIVIKLREHFGNKTIKFNQSSGGDPEIVRHSF
NCGGEFFYCDTTQLFNSTWNGTEGNNTGNSTITLPCRKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
LLTRDGGTEGNGTENETEIFRPGGGDMRDNRSELYKYKVVKVEPLGVAPTRAKRRVVQRMGARASVLSGG
ELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLETSEGCRQILGQLQPSLQTGSEELRSLYNTV
ATLYCVHQRIEIKDTKEALDKIEEEQNKSKKKAQQAADTGHSNQVSQNYPIVQNIQGQMVHQAI SPRTL N
AWVKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAMQMLKETINEEAAEWDRVHPVHAGPIA
PGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGLNKIVRMYSPITSILDIRQGPKEPFRD
YVDRFYKTLRAEQASQEVKNWMTETLLVQNANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLMGK
WSKSSVVGWPTVRERMRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQEEEEVGFVPVTPQVP
LRPMTYKAAVDLSHFLKEKGLEGLIHSQRRQDILDWLYHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLV
PVEPDKVEEANKGENTSAAHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEP
WKHPGSQPKTACTNCYCKKCCFHCQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSKGEP
TGPKE [SEQ ID NO: 79]

Figure 19

pRix46



DNA sequence of insert

```

ATGGCCGAGCAGCTGTGGGTACCGTCTACTACGGCGTGCCTGTGTGGAAGGAGGCCACGACCACCTCTT
CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
CGGACCCCAACCCCAAGGAGGTGGTGTGTTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
GTGGATCAGATGCACGAGGACATCATCTCTGTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
CCGGAGAGATTTCGGAAGGGCGAGATCAAGAAGTGTCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
CAAGACGACGCGTAATTTTCACTACTGCAACAGCAGCGTCATGACGCGAGGCCTGCCCCAAGGTGT
CCTTCGAACCAATCCCGATCCATTACTGTGCCCCCTGCCGGATTTCGCGATCCTCAAGTGTAACAACAAGACC
TTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCCGCCCCGTCGTGAG
CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGTATCCGGTCGGACAACCTTCATGGACA
ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAAACAACACC
CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCCGCAAGATCATCGGCGACATCCGGCA
GGCCCATTTGCAACCTCTCCCGCGCCCAAGTGAATAACACCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
ACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCAGGG
CAACAACACAGAGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAAACATCACCGGCCTG
CTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGGAGAACGAGACGGAGATCTTCAGGCCCGGCGG
CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
TGGCCCCCACC CGGGCCAAGCGCCGCTCGTGCAGAGAATGGGTGCCCGAGCTTCGGTACTGTCTGGTGGGA
GAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCCGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGT
GTGGGCCTCGAGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGTCGCC
AGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGGTCCCTTGTATAACACAGTG
GCTACCCTCTACTGCGTACACCAGAGGATCGAGATTAAGGATACCAAGGAGGCCTTGGACAAAATTGAGGA
GGAGCAAAAACAAGAGCAAGAAGAAGGCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCAC
AGAACTATCCTATTGTCCAAAACATTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCCGGACGCTCAAT
GCCTGGGTGAAGGTTGTGCAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCATGTTCTCCGCTTTGAGTGA
GGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGGCCGCCATGCAATGT
  
```

Figure 19 continued

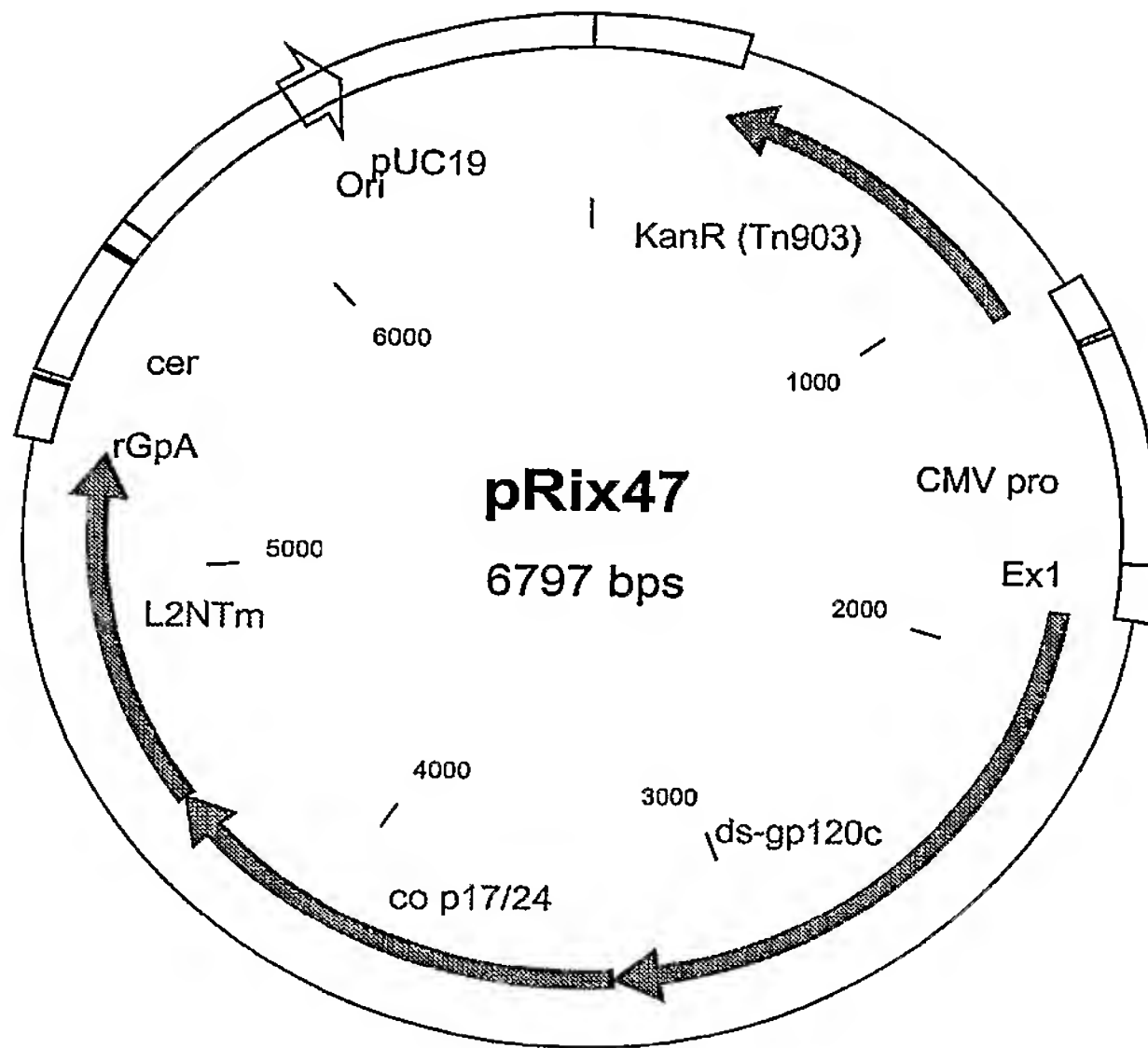
TGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCG
 CCCGGACAGATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAATCGG
 ATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGGATCATTTCTCGGTCTCAATA
 AAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAAGAGCCTTTTAGGGAT
 TACGTCGACCGGTTTTATAAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAACCTGGATGACGGA
 GACACTCCTGGTACAGAACGCTAACCCCGACTGCAAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACCC
 TGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCGGACCCGGACACAAAGCCAGAGTGTTGATGGGCAAG
 TGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCAGCAGCAGA
 TGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCA
 ATGCTGCTTGTGCCTGGCTAGAAGCACAAAGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCT
 TTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAGAAAAGGGGGGACTGGAAGG
 GCTAATTCCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTG
 ATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTA
 CCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCGCCTTACACCCTGTGAGCCT
 GCATGGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCACG
 TGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTAGAGCCC
 TGGAAGCATCCAGGAAGTCAGCCTAAAACCTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTTCATTG
 CCAAGTTTGT'TTCATAACAGCTGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGAC
 CTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCAAAGGGGAGCCG
 ACAGGCCCGAAGGAATAA [SEQ ID NO: 80]

Aminoacid sequence of insert

MAEQLWVTVYYGVVPVWKEATTTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVVLGNVTEYFNMWKNM
 VDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNTSTTTTNSNGWTGEIRKGEIKNCSFNITTSIRDKV
 QKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNKKT
 FDGKGLCTNVSTVQCTHGIRPVVSTQLLLNGLSLAEEEVVIRSDNFMDNTKTIIVQLNESVAINCTRPNNT
 RKGIIHIGPGRAFYAARKIIGDIRQAHCNLSRAQWNNTLKQIVIKLREHFGNKTIFKNQSSGGDPEIVRHSF
 NCGGEFFYCDTTQLFNSTWNGTEGNNTEGNSTITLPCRIKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
 LLTRDGGTEGNGTENETEIFRPGGGDMRDNRSELYKYKVVKVEPLGVAPTRAKRRVVQRMGARASVLSGG
 ELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLETSEGCRQILGQLQPSLQTGSEELRSLYNTV
 ATLYCVHQRIEIKDTKEALDKIEEEQNKSKKKAQQAADTGHSNQVSQNYPIVQNIQGQMVHQAI SPRTLN
 AWWKVVEEKAFSPEVIPMFSALSEGATPQDLNLTMLNTVGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIA
 PGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGLNKIVRMYSPITSILDIRQGPKEPFRD
 YVDRFYKTLRAEQASQEVKNWMTETLLVQNaNPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLMGK
 WSKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQEEEEVGFVPTPQVP
 LRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWLYHTQGYFPDWQNYTPGPGVRYPLTFGW CYKLV
 PVEPDKVEEANKGENTSALHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEP
 WKHPGSQPKTACTNCYCKKCCFHCQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSKGEP
 TGPKE [SEQ ID NO: 81]

Figure 20

pRix47

**DNA sequence of insert**

ATGGCCGAGCAGCTGTGGGTCACCGTCTACTACGGCGTGCCTGTGTGGAAGGAGGCCACGACCACCCTCTT
 CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
 CGGACCCCAACCCCAAGGAGGTGGTGTGCTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTTCGAAGGGCGAGATCAAGAAGTCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTTCAGACTCATTCACTGCAACAGCAGCGTCATGACGACGAGCCTGCCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCCGCGGATTTCGCGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCCGCCCCGTCGTGAG
 CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGTATCCGGTTCGGACAACCTTCATGGACA
 ACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCCGCAAGATCATCGGCGACATCCGGCA
 GGCCCATTTGCAACCTCTCCCGCGCCAGTGGAATAACACCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCGAGGG
 CAACAACACAGAGGGGAAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTG
 CTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCACCCGGGCCAAGCGCCGCTCGTGCAGAGAATGGGTGCCCGAGCTTCGGTACTGTCTGGTGGGA
 GAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCCGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGT
 GTGGGCCTCGAGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGTCGCC
 AGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGGTCCTTGTATAACACAGTG
 GCTACCTCTACTGCGTACACCAGAGGATCGAGATTAAGGATACCAAGGAGGCCTTGGACAAAATTGAGGA
 GGAGCAAAACAAGAGCAAGAAGAAGGCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCAC
 AGAACTATCCTATTGTCCAAAACATTCAGGGCCAGATGGTTTCATCAGGCCATCAGCCCCCGGACGCTCAAT
 GCCTGGGTGAAGGTTGTGCAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCCATGTTCTCCGCTTTGAGTGA
 GGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGGCCGCCATGCAAATGT

Figure 20 continued

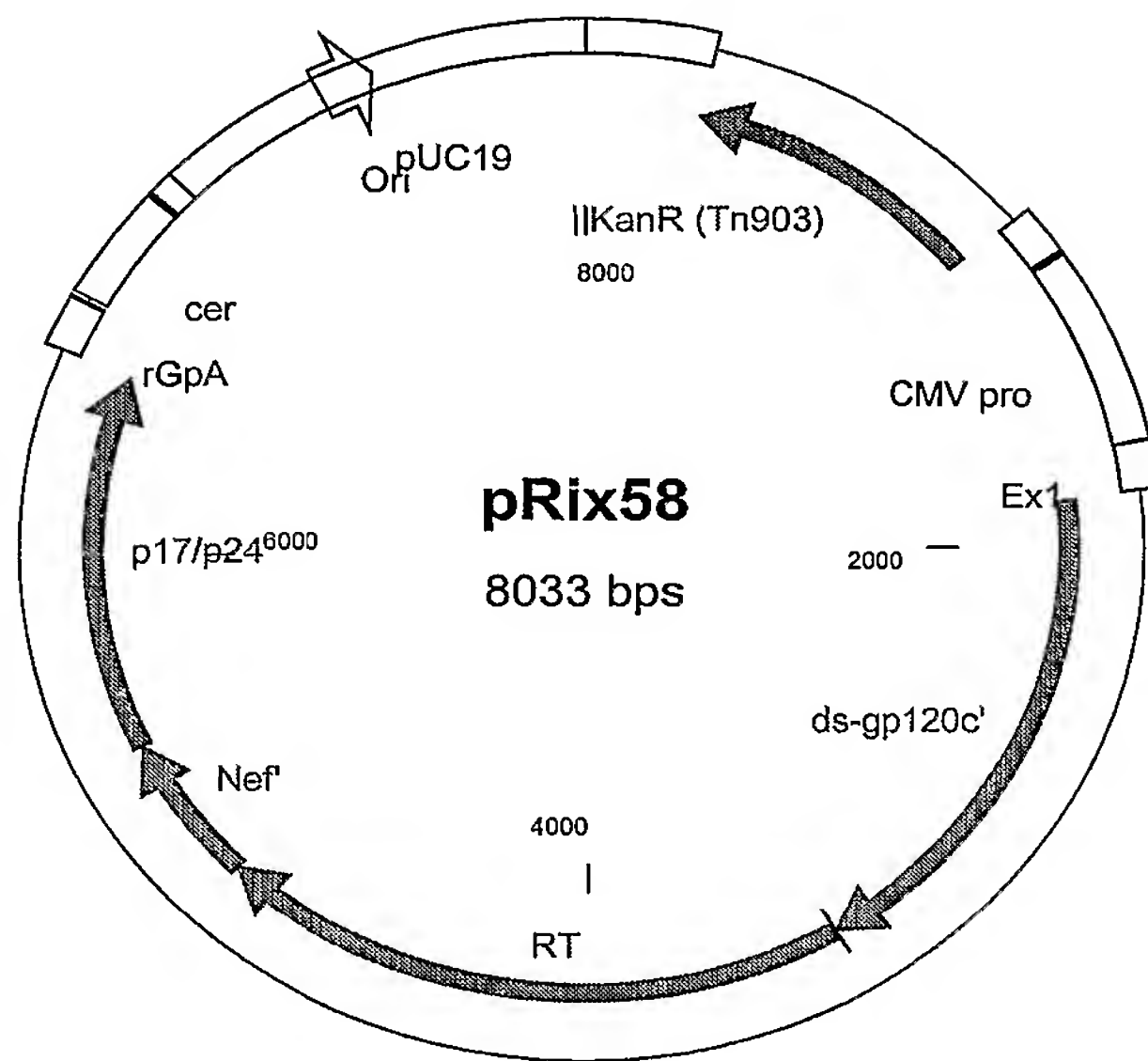
TGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCG
CCCGGACAGATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAATCGG
ATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGGATCATTCTCGGTCTCAATA
AAATTGTTAGAATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAAGAGCCTTTTAGGGAT
TACGTCGACCGGTTTTATAAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAACCTGGATGACGGA
GACACTCCTGGTACAGAACGCTAACCCCGACTGCAAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACCC
TGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCGGACCCGGACACAAAGCCAGAGTGTTGATGGGCAAG
TGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCAGCAGCAGA
TGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCA
ATGCTGCTTGTGCCTGGCTAGAAGCACAAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCT
TTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGGGGACTGGAAGG
GCTAATTCACCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTG
ATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTA
CCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGGCACACCCTGTGAGCCT
GCATGGAATGGATGACCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCAGC
TGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTAGAGCCC
TGGAAGCATCCAGGAAGTCAGCCTAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTGCTTTTCATTG
CCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGAC
CTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCAAAGGGGAGCCG
ACAGGCCCGAAGGAATAA [SEQ ID NO: 82]

Aminoacid sequence of insert

MAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVVLGNVTEYFNMWKNNM
VDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNT'NSTTTTSSNGWTGEIRKGEIKNCSFNITTSIRDKV
QKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNNKT
FDGKGLCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSDNFMDNTKTIIVQLNESVAINCTRPNNNT
RKGIIHIGPGRFYAARKIIGDIRQAHCNLSRAQWNNTLKQIVIKLREHFGNKTIFKNQSSGGDPEIVRHSF
NCGGEFFYCDTTQLFNSTWNGTEGNNTTEGNSTITLPCRIKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
LLTRDGGTEGNGTENETEIFRPGGGDMRDNRSELYKYKVVKVEPLGVAPTRAKRRVVQRMGARASVLSGG
ELDRWEKIRLRPGGKKKYKLKHIIVWASRELERFAVNPGLLETSEGCRQILGQLQPSLQTGSEELRSLYNTV
ATLYCVHQRIEIKDTKEALDKIEEEQNKSKKKAQQAADTGHSNQVSQNYPIVQNIQGQMVHQAISPRTLN
AWKVVEEKAFSPEVIPMFSALSEGATPQDLNMTLNTVGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIA
PGQMREPRGSDIAGTTSTLQEQIGWMTNPNPIPVGGEIYKRWIILGLNKIVRMYSPTSILDIRQGPKEPFRD
YVDRFYKTLRAEQASQEVKNWMTETLLVQANANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLMGK
WSKSSVVGWPTVRERMRRRAEPAADGVGAASRDLEKHGAITSNTAATNAACAWLEAQEEEEVGFPVTPQVP
LRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWLYHTQGYFPDWQNYTPGPGVRYPLTFGWICYKLV
PVEPDKVEEANKGENTSLAHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEP
WKHPGSQPKTACTNCYCKKCCFHCQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSKGEP
TGPKE [SEQ ID NO: 83]

Figure 21

pRix58

**DNA sequence of insert**

ATGGCCGAGCAGCTGTGGGTACCGTCTACTACGGCGTGCCTGTGTGGAAGGAGGCCACGACCACCCTCTT
 CTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCGTGCCTA
 CGGACCCCAACCCCAAGGAGGTGGTGTCTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAATAACATG
 GTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACGCC
 TCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACGGCTGGA
 CCGGAGAGATTTCGGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCACGACCTCGATCAGAGACAAGGTG
 CAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCACCAAGAA
 CAAGACGACGCGTAATTTTCAGACTCATTCACTGCAACAGCAGCGTCATGACGCAGGCCTGCCCCAAGGTGT
 CCTTCGAACCAATCCCGATCCATTACTGTGCCCTTGCCGGATTCGCGATCCTCAAGTGTAACAACAAGACC
 TTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCCGCCCCGTCTGTGAG
 CACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGATCCGGTCGGACAACCTTCATGGACA
 ACACCAAGACAATCATCTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAACAACACC
 CGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCGCAAGATCATCGGCGACATCCGGCA
 GGCCCATTGCAACCTCTCCCGCGCCCAAGTGGGAATAACACCCTGAAGCAGATCGTGATCAAGCTGAGAGAGC
 ACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCACTCCTTC
 AACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCACCGAGGG
 CAACAACACAGAGGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
 AGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACCGGCCTG
 CTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCCCCGGCGG
 CGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGCTCGGCG
 TGGCCCCCACC CGGGCAAGCGCCGCGTCTGTGAGAGAATGGGCCCCATCAGTCCCATCGAGACCGTGCCG
 GTGAAGCTGAAACCCGGGATGGACGGCCCCAAGGTCAAGCAGTGGCCACTCACCGAGGAGAAGATCAAGGC
 CCTGGTGGAGATCTGCACCGAGATGGAGAAAGAGGGCAAGATCAGCAAGATCGGGCCTGAGAACCATAACA
 ACACCCCGTGTTTGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGATTTCGGGAGCTG
 AATAAGCGGACCCAGGATTTCTGGGAGGTCCAGCTGGGCATCCCCCATCCGGCCGGCCTGAAGAAGAAGAA
 GAGCGTGACCGTGCTGGACGTGGGCGACGCTTACTTCAGCGTCCCTCTGGACGAGGACTTTAGAAAGTACA
 CCGCCTTTACCATCCCATCTATCAACAACGAGACCCCTGGCATCAGATATCAGTACAACGTCCTCCCCCAG
 GGCTGGAAGGGCTCTCCCGCCATTTTCCAGAGCTCCATGACCAAGATCCTGGAGCCGTTTCGGAAGCAGAA
 CCCCAGATATCGTCATCTACCAGTACATGGACGACCTGTACGTGGGCTCTGACCTGGAAATCGGGCAGCATC

Figure 21 continued

GCACGAAGATTGAGGAGCTGAGGCAGCATCTGCTGAGATGGGGCCTGACCACTCCGGACAAGAAGCATCAG
 AAGGAGCCGCCATTCTGAAGATGGGCTACGAGCTCCATCCCGACAAGTGGACCGTGCAGCCTATCGTCCT
 CCCCAGAGAAGGACAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTCAACTGGGCTAGCCAGA
 TCTATCCCGGGATCAAGGTGCGCCAGCTCTGCAAGCTGCTGCGCGGCACCAAGGCCCTGACCGAGGTGATT
 CCCCTCACGGAGGAAGCCGAGCTCGAGCTGGCTGAGAACCGGGAGATCCTGAAGGAGCCCGTGCACGGCGT
 GTACTATGACCCCTCCAAGGACCTGATCGCCGAAATCCAGAAGCAGGGCCAGGGGCAGTGGACATACCAGA
 TTTACCAGGAGCCTTTCAAGAACCTCAAGACCGGCAAGTACGCCCGCATGAGGGGCGCCACACCAACGAT
 GTCAAGCAGCTGACCGAGGCCGTCCAGAAGATCACGACCGAGTCCATCGTGATCTGGGGGAAGACACCCAA
 GTTCAAGCTGCCTATCCAGAAGGAGACCTGGGAGACGTGGTGGACCGAATATTGGCAGGCCACCTGGATT
 CCGAGTGGGAGTTCTGAATACACCTCCTCTGGTGAAGCTGTGGTACCAGCTCGAGAAGGAGCCCATCGTG
 GCGCGGAGACATTTACGTGGACGGCGCGGCCAACCGCGAAACAAAGCTCGGGAAGGCCGGGTACGTAC
 CAACCGGGGCGCCAGAAGGTGCTACCCCTGACCGACACCACCAACCAGAAGACGGAGCTGCAGGCCATCT
 ATCTCGCTCTCCAGGACTCCGGCCTGGAGGTGAACATCGTGACGGACAGCCAGTACGCGCTGGGCATTATT
 CAGGCCCAGCCGGACAGTCCGAGAGCGAACTGGTGAACCAGATTATCGAGCAGCTGATCAAGAAAGAGAA
 GGTCTACCTCGCCTGGGTCCCGGCCCATAGGGCATTGGCGGCAACGAGCAGGTGACAAAGCTGGTGAGTG
 CGGGGATTAGAAAGGTGCTGATGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTAC
 AAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAGAAAAGGGGGGACTGGAAGGGCTAATCACTCCCAAAG
 AAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACAC
 CAGGGCCAGGGGTGAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAG
 GTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCTGTGAGCCTGCATGGGATGGATGACCC
 GGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCACGTGGCCCGAGAGCTGCATC
 CGGAGTACTTCAAGAACTGCATGGGTGCCCCGAGCTTCGGTACTGTCTGGTGGAGAGCTGGACAGATGGGAG
 AAAATTAGGCTGCGCCCCGGGAGGCAAAAAGAAATACAAGCTCAAGCATATCGTGTGGGCCTCGAGGGAGCT
 TGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACATCTGAGGGATGTCGCCAGATCCTGGGGCAATTGC
 AGCCATCCCTCCAGACCGGAGTGAAGAGCTGAGGTCTTGTATAACACAGTGGCTACCCTCTACTGCGTA
 CACCAGAGGATCGAGATTAAGGATACCAAGGAGGCCTTGGAACAAATGAGGAGGAGCAAAACAAGAGCAA
 GAAGAAGGCCCCAGCAGGCAGCTGCTGACACTGGGCATAGCAACCAGGTATCACAGAACTATCCTATTGTCC
 AAAACATTCAGGGCCAGATGGTTCATCAGGCCATCAGCCCCCGGACGCTCAATGCCTGGGTGAAGGTTGTC
 GAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCCATGTTCTCCGCTTTGAGTGAGGGGGCCACTCCTCAGGA
 CCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGGCCGCCATGCAAATGTTGAAGGAGACTATCAACG
 AGGAGGCAGCCGAGTGGGACAGAGTGCATCCCGTCCACGCTGGCCCAATCGCGCCCCGACAGATGCGGGAG
 CCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACTGCAAGAGCAAATCGGATGGATGACCAACAATCC
 TCCCATCCCAGTTGGAGAAATCTATAACGGTGGATCATCCTGGGCCTGAACAAGATCGTGCGCATGTACT
 CTCCGACATCCATCCTTGACATTAGACAGGGACCCAAAGAGCCTTTTAGGGATTACGTGACCGGTTTTAT
 AAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAAAAACCTGGATGACGGAGACACTCCTGGTACAGAA
 CGCTAACCCCGACTGCAAAACAATCTTGAAGGCACTAGGCCCGGCTGCCACCCTGGAAGAGATGATGACCG
 CCTGTCAGGGAGTAGGCGGACCCGGACACAAAGCCAGAGTGTGTAA [SEQ ID NO: 84]

Aminoacid sequence of insert

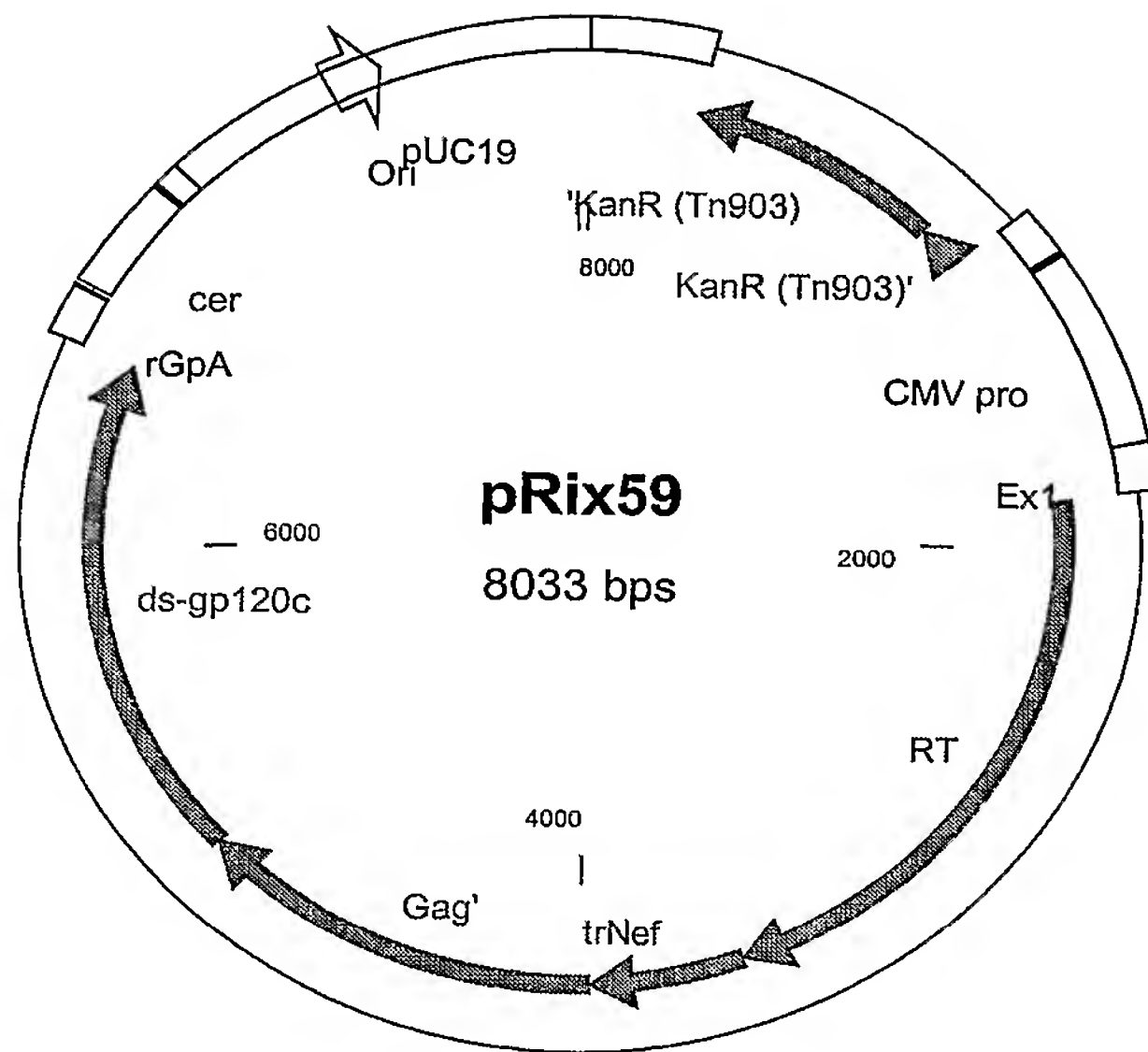
MAEQLWVTVYYGVVPVWKEATTTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVVLGNVTEYFNMWKNM
 VDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTTNSNGWTGEIRKGEIKNCSFNITTSIRDKV
 QKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNKKT
 FDGKGLCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSDNFMDNTKTIIVQLNESVAINCTRPNNNT
 RKGIIHIGPGRIFYAARKIIGDIRQAHCNLSRAQWNNTLQKQIVIKLREHFGNKTIFNQSSGGDPEIVRHSF
 NCGGEFFYCDTTQLFNSTWNGTEGNTEGNSTITLPCRIKQIINMWQEVGKAMYAPPIGGQIRCSSNITGL
 LLTRDGGTEGNTEGNETEIFRPGGGDMRDNRSELYKYKVVKVEPLGVAPTRAKRRVVQRMGPISPIETVP
 VKLKPGMDGPKVKQWPLTEEKIKALVEICTEMEKEGKISKIGPENPYNTPVFAIKKKDSTKWRKLVDREL
 NKRTQDFWEVQLGIPHPAGLKKKKSVTVLVDVGDAYFSVPLDEDFRKYTAFTIPSINNETPGIRYQYNVLPQ
 GWKGSPAIFQSSMTKILEPFRKQNPDIVIYQYMDDLIVGSDLEIGQHRTKIEELRQHLLRWGLTTPDKKHQ
 KEPPFLKMGYELHPDKWTVQPIVLPEKDSWTVNDIQKLVGKLNWASQIYPGIKVRQLCKLLRGTKALTEVI
 PLTEEALELELAENREILKEPVHGVYDPSKDLIAEIQKQGQGWQTYQIYQEPFKNLKTGKYARMRGAHTND
 VKQLTEAVQKITTESIVIWGKTPKFKLPQKETWETWTEYWQATWIPWEFEVNTPLVLKLWYQLEKEPIV
 GAETFYVDGAANRETKLGKAGYVTNRGRQKVVTLTDTTNQKTELQAIYLAALQDSGLEVNIVTDSQYALGII

Figure 21 continued

QAQPDQSESELVNQIIEQLIKKEKVYLAWVPAHKGIGGNEQVDKLVSAGIRKVL MVGFPVTPQVPLRPMTY
KAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHTQGYFPDWQNYTPGPGVRYPLTFGW CYKLV PVEPDK
VEEANKGENTSLLHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCMGARASVLSGGELDRWE
KIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLETSEGCRQILGQLQPSLQTGSEELRSLYNTVATLYCV
HQRIEIKDTKEALDKIEEEQNKSKKKAQQAAADTGHSNQVSQNYPIVQNIQGQMVHQ AISPRTLNAWVKVV
EEKAFSPEVIPMFSALSEGATPQDLN TMLNTVGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIAPGQMRE
PRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWII LGLNKIVRMYSPTSILDIRQGPKEPFRDYVDRFY
KTLRAEQASQEVKNWMTETLLVQNANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVL [SEQ ID
NO: 85]

Figure 22

pRix59



DNA sequence of insert

ATGGGCCCCATCAGTCCCATCGAGACCGTGCCGGTGAAGCTGAAACCCGGGATGGACGGCCCCAAGGTCAA
 GCAGTGGCCACTCACCGAGGAGAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAAGAGGGCA
 AGATCAGCAAGATCGGGCCGGAGAACCATAACAACCCCCGTGTTTGCCATCAAGAAGAAGGACAGCACC
 AAGTGGCGCAAGCTGGTGGATTTCCGGGAGCTGAATAAGCGGACCCAGGATTTCTGGGAGGTCCAGCTGGG
 CATCCCCCATCCGGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGCTGGACGTGGGCGACGCTTACTTCA
 GCGTCCCTCTGGACGAGGACTTTAGAAAGTACACCGCCTTTACCATCCCATCTATCAACAACGAGACCCCT
 GGCATCAGATATCAGTACAACGTCCTCCCCCAGGGCTGGAAGGGCTCTCCCGCCATTTTCCAGAGCTCCAT
 GACCAAGATCCTGGAGCCGTTTTCGGAAGCAGAACCCCGATATCGTCATCTACCAGTACATGGACGACCTGT
 ACGTGGGCTCTGACCTGGAAATCGGGCAGCATCGCACGAAGATTGAGGAGCTGAGGCAGCATCTGCTGAGA
 TGGGGCCTGACCACTCCGGACAAGAAGCATCAGAAGGAGCCGCCATTCCTGAAGATGGGCTACGAGCTCCA
 TCCCGACAAGTGGACCGTGACGCTATCGTCCTCCCCGAGAAGGACAGCTGGACCGTGAACGACATCCAGA
 AGCTGGTGGGCAAGCTCAACTGGGCTAGCCAGATCTATCCCGGGATCAAGGTGCGCCAGCTCTGCAAGCTG
 CTGCGCGGCACCAAGGCCCTGACCGAGGTGATTCCCCTCACGGAGGAAGCCGAGCTCGAGCTGGCTGAGAA
 CCGGGAGATCCTGAAGGAGCCCGTGACGCGGTGTACTATGACCCCTCCAAGGACCTGATCGCCGAAATCC
 AGAAGCAGGGCCAGGGGCAGTGGACATACCAGATTTACCAGGAGCCTTTCAAGAACCTCAAGACCGGCAAG
 TACGCCCCGATGAGGGGCGCCACACCAACGATGTCAAGCAGCTGACCGAGGCGGTCCAGAAGATCACGAC
 CGAGTCCATCGTGATCTGGGGGAAGACACCCAAGTTCAAGCTGCCTATCCAGAAGGAGACCTGGGAGACGT
 GGTGGACCGAATATTGGCAGGCCACCTGGATTCCCGAGTGGGAGTTCTGTAATACACCTCCTCTGGTGAAG
 CTGTGGTACCAGCTCGAGAAGGAGCCCATCGTGGGCGCGGAGACATTTCTACGTGGACGGCGCGGCCAACC
 CGAAACAAAGCTCGGGGAAGGCCGGGTACGTCACCAACCGGGGCCGAGAGGTCTGTCACCCCTGACCGACA
 CCACCAACCAGAAGACGGAGCTGCAGGCCATCTATCTCGCTCTCCAGGACTCCGGCCTGGAGGTGAACATC
 GTGACGGACAGCCAGTACGCGCTGGGCATTATTCAGGCCCAGCCGACAGTCCGAGAGCGAACTGGTGAA
 CCAGATTATCGAGCAGCTGATCAAGAAAGAGAAGGTCTACCTCGCTGGGTCCCGGCCATAAGGGCATTTG
 GCGGCAACGAGCAGGTGACAAAGCTGGTGAAGTGCAGGGGATTAGAAAGGTGCTGATGGTGGGTTTTCCAGTC
 ACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTAAAGAAAA
 GGGGGGACTGGAAGGGCTAATTCCTCCCAAAGAAGACAAGATATCCTTGATCTGTGGATCTACCACACAC
 AAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACCTTTGGATGG
 TGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTT
 ACACCCTGTGAGCCTGCATGGGATGGATGACCCGGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCC

Figure 22 continued

TAGCATTTCATCACGTGGCCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCATGGGTGCCCCGAGCTTCG
 GTACTGTCTGGTGGAGAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCCGGGAGGCAAAAAGAAATACAA
 GCTCAAGCATATCGTGTGGGCCTCGAGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACAT
 CTGAGGGATGTCGCCAGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGGTCC
 TTGTATAACACAGTGGCTACCCTCTACTGCGTACACCAGAGGATCGAGATTAAGGATACCAAGGAGGCCTT
 GGACAAAATTGAGGAGGAGCAAAACAAGAGCAAGAAGAAGGCCCCAGCAGGCAGCTGCTGACACTGGGCATA
 GCAACCAGGTATCACAGAACTATCCTATTGTCCAAAACATTTCAGGGCCAGATGGTTTCATCAGGCCATCAGC
 CCCCCGACGCTCAATGCCTGGGTGAAGGTTGTCTGAAGAGAAGGCCCTTTTCTCCTGAGGTTATCCCCATGTT
 CTCCGCTTTGAGTGAGGGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGG
 CCGCCATGCAAATGTTGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCATCCCGTCCAC
 GCTGGCCCAATCGCGCCCCGACAGATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACT
 GCAAGAGCAAATCGGATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGGATCA
 TCCTGGGCCTGAACAAGATCGTGCGCATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAA
 GAGCCTTTTAGGGATTACGTGACCGGTTTTATAAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAA
 AACTGGATGACGGAGACACTCCTGGTACAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCACTAG
 GCCCCGCTGCCACCCTGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCGGACCCCGACACAAAGCCAGA
 GTGTTGATGGCCGAGCAGCTGTGGGTACCGTCTACTACGGCGTGCTGTGTGGAAGGAGGCCACGACCAC
 CCTCTTCTGCGCGAGCGACGCCAAGGCCTACGACACGGAAGTGCATAACGTGTGGGCGACGCATGCTTGCG
 TGCCTACGGACCCCAACCCCCAGGAGGTGGTGCTGGGAAACGTGACCGAGTACTTCAACATGTGGAAGAAT
 AACATGGTGGATCAGATGCACGAGGACATCATCTCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCT
 GACGCCTCTCTGCGTGACACTGGACTGTGACGACGTCAACACCACCAACAGCACTACCACCACCAGCAACG
 GCTGGACCGGAGAGATTTCGAAGGGCGAGATCAAGAACTGCTCCTTCAATATCACGACCTCGATCAGAGAC
 AAGGTGCAGAAGGAATACGCGCTGTTTTATAATCTCGATGTGGTCCCCATCGACGACGACAATGCCACCAC
 CAAGAACAAGACGACGCGTAATTTTCAGACTCATTCACTGCAACAGCAGCGTCATGACGCAGGCCTGCCCCA
 AGGTGTCCTTCGAACCAATCCCGATCCATTACTGTGCCCTGCCGGATTGCGGATCCTCAAGTGTAACAAC
 AAGACCTTCGACGGGAAGGGCCTGTGCACCAACGTGACGACGGTGCAGTGCACCCATGGCATCCGCCCCGT
 CGTGAGCACCCAGCTGCTGCTGAACGGGTCCCTGGCTGAGGAGGAGGTGGTGATCCGGTCGGACAACCTTCA
 TGGACAACACCAAGACAATCATCGTCCAGCTGAACGAGTCTGTGGCGATTAACTGTACCCGGCCTAACAAAC
 AACACCCGTAAGGGCATCCACATCGGGCCTGGACGGGCCTTCTATGCCGCCCCGCAAGATCATCGGCGACAT
 CCGGCAGGCCCATTTGCAACCTCTCCGCGCCCAAGTGGAAATAACACCCTGAAGCAGATCGTGATCAAGCTGA
 GAGAGCACTTTGGAAACAAGACCATCAAGTTCAATCAGAGTTCTGGCGGAGACCCCGAGATCGTGCGGCAC
 TCCTTCAACTGCGGGGGCGAGTTCTTCTACTGCGATACGACACAGCTCTTCAACTCCACCTGGAACGGCAC
 CGAGGGCAACAACACAGAGGGGAACTCCACTATCACCTCCCTTGCCGCATCAAGCAGATCATCAACATGT
 GGCAGGAGGTGGGAAAGGCCATGTATGCCCCCCCCATCGGGGGCCAGATCCGCTGCTCCTCCAACATCACC
 GGCCTGCTGCTCACCAGAGACGGGGGCACCGAGGGCAACGGCACGGAGAACGAGACGGAGATCTTCAGGCC
 CGGCGGCGGCGACATGAGGGATAACTGGCGGAGCGAGCTGTACAAGTACAAGGTGGTGAAGGTGGAGCCGC
 TCGGCGTGGCCCCCACCCGGGCCAAGCGCCGCGTCTGTCAGAGATGA [SEQ ID NO: 86]

Aminoacid sequence of insert

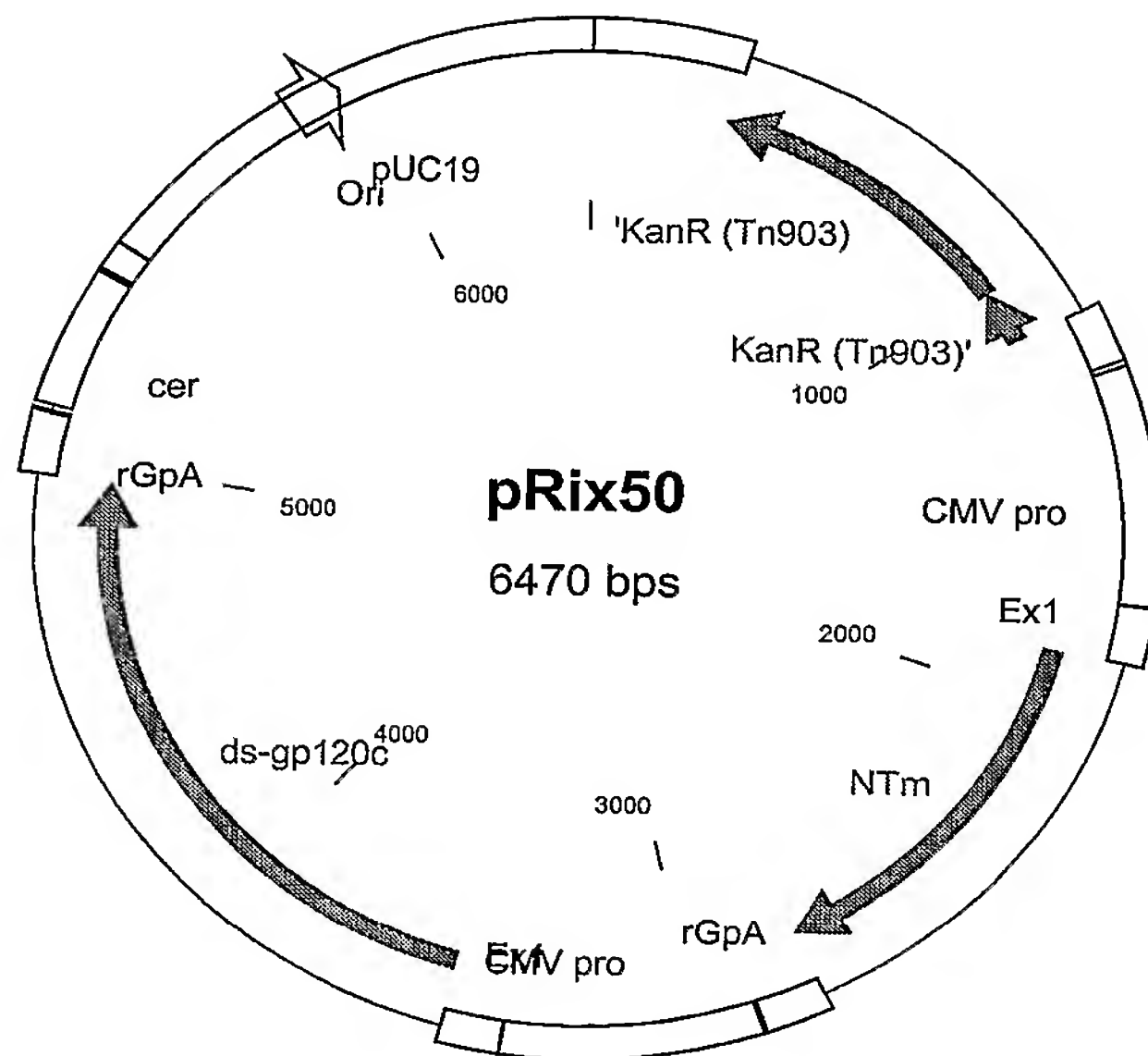
MGPISPIETVPVKLKPMDGPKVKQWPLTEEKIKALVEICTEMEKEGKISKIGPENPYNTPVFAIKKKDST
 KWRKLVDFRELNKRTQDFWEVQLGIPHPAGLKKKKSVTVLVDVGDAYFSVPLDEDFRKYTAFTIPSINNETP
 GIRYQYNVLPQGWKGSPAI FQSSMTKILEPFRKQNPDIVIYQYMDDLVVGSDLEIGQHRTKIEELRQHLLR
 WGLTTPDKKHQKEPPFLKMGYELHPDKWTVQPIVLPEKDSWTVNDIQKLVGKLNWASQIYPGIKVRQLCKL
 LRGTKALTEVIPLTEEALELAENREILKEPVHGVYYDPSKDLIAEIQKQGQGWTYQIYQEPFKNLKTGK
 YARMRGAHTNDVKQLTEAVQKITTESIVIWGKTPKFKLPIQKETWETWWTEYWQATWIPEWEFVNTPLVK
 LWYQLEKEPIVGAETFYVDGAANRETKLGKAGYVTNRGRQKVVTLTDTTNQKTELQAIYLALQDSGLEVNI
 VTDSQYALGIIQAQPDQSESELVNQIIIEQLIKKEKVYLAWVPAHKGIGGNEQVDKLVSAGIRKVLMMVGFV
 TPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWIYHTQGYFPDWQNYTPGPGVRYPLTFGW
 CYKLVPVEPDKVEEANKGENTSLHHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCMGARAS
 VLSGGELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLETSEGCRQILGQLQPSLQTGSEELRS
 LYNTVATLYCVHQRIEIKDTKEALDKIEEEQNKSKKKAQQAADTGHSNQVSQNYPIVQNIQGQMVHQAI
 PRTLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNMTLNTVGGHQAAMQMLKETINEEAAEWDRVHPVH
 AGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGLNKIVRMYSPTSILDIRQGP
 EPFRDYVDRFYKTLRAEQASQEVKNWMTETLLVQNANPDCKTILKALGPAATLEEMMTACQGVGGPGHKAR

Figure 22 continued

VLMAEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEVVLGNVTEYFNMWKN
NMVDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTTSNGWTGEIRKGEIKNCSFNITTSIRD
KVQKEYALFYNLDVVPIDDDNATTKNKTTRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNN
KTFDGKGLCTNVSTVQCTHGIRPVVSTQLLNGSLAEEEVVIRSDNFMNDNTKTIIVQLNESVAINCTRPNN
NTRKGIHIGPGRAFYAARKIIGDIRQAHCNLSRAQWNNTLKQIVIKLREHFGNKTIKFNQSSGGDPEIVRH
SFNCGGEFFYCDTTQLFNSTWNGTEGNNTEGNSTITLPCRIKQIINMWQEVGKAMYAPPIGGQIRCSSNIT
GLLLTRDGGTEGNGTENETEIFRPGGGDMRDNRSELYKYKVVKVEPLGVAPTRAKRRVVQR [SEQ ID
NO: 87]

Figure 23

pRix50

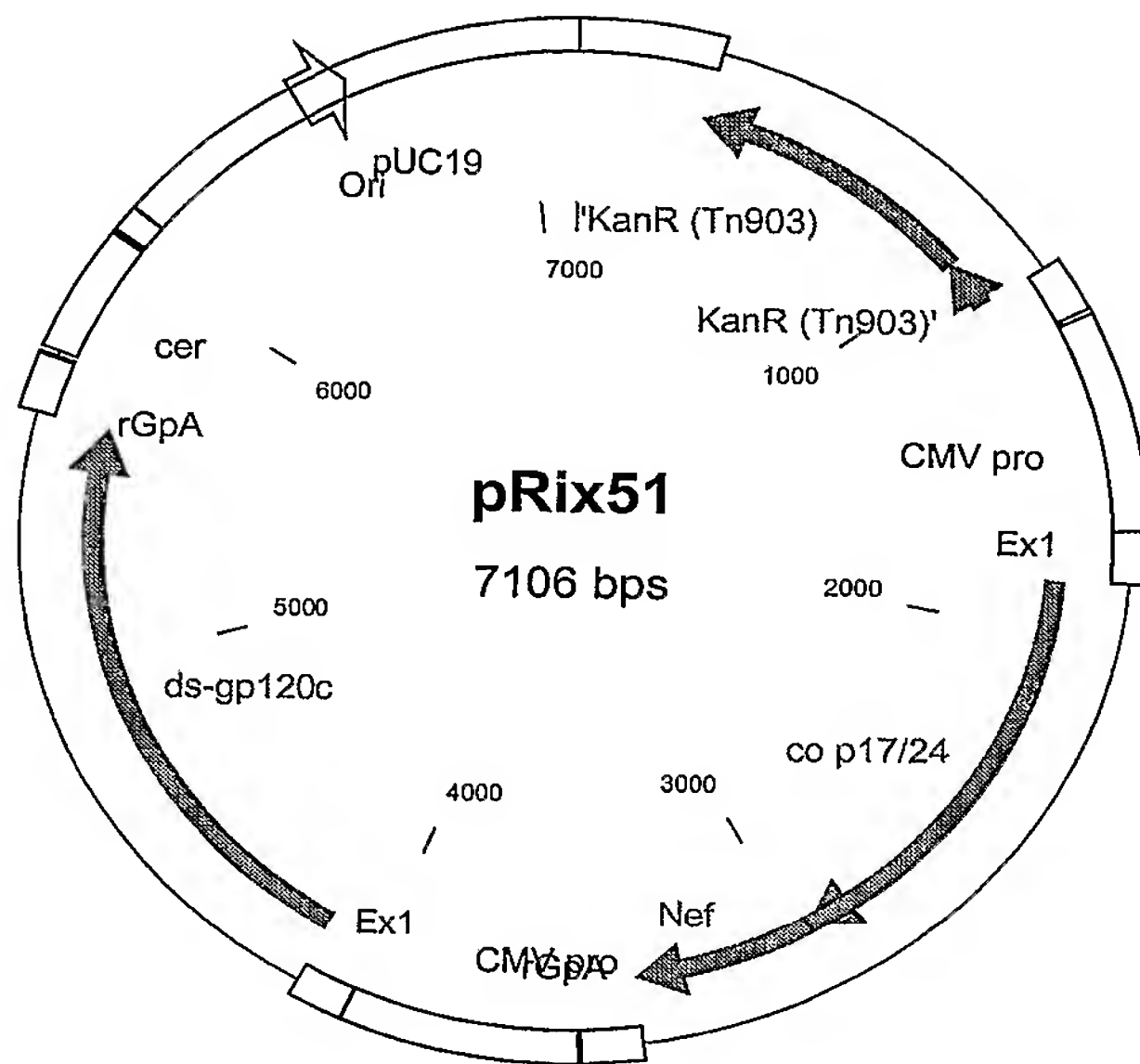


DNA and amino acid sequences of inserts:

Identical to pNTm and pRix12

Figure 24

pRix51

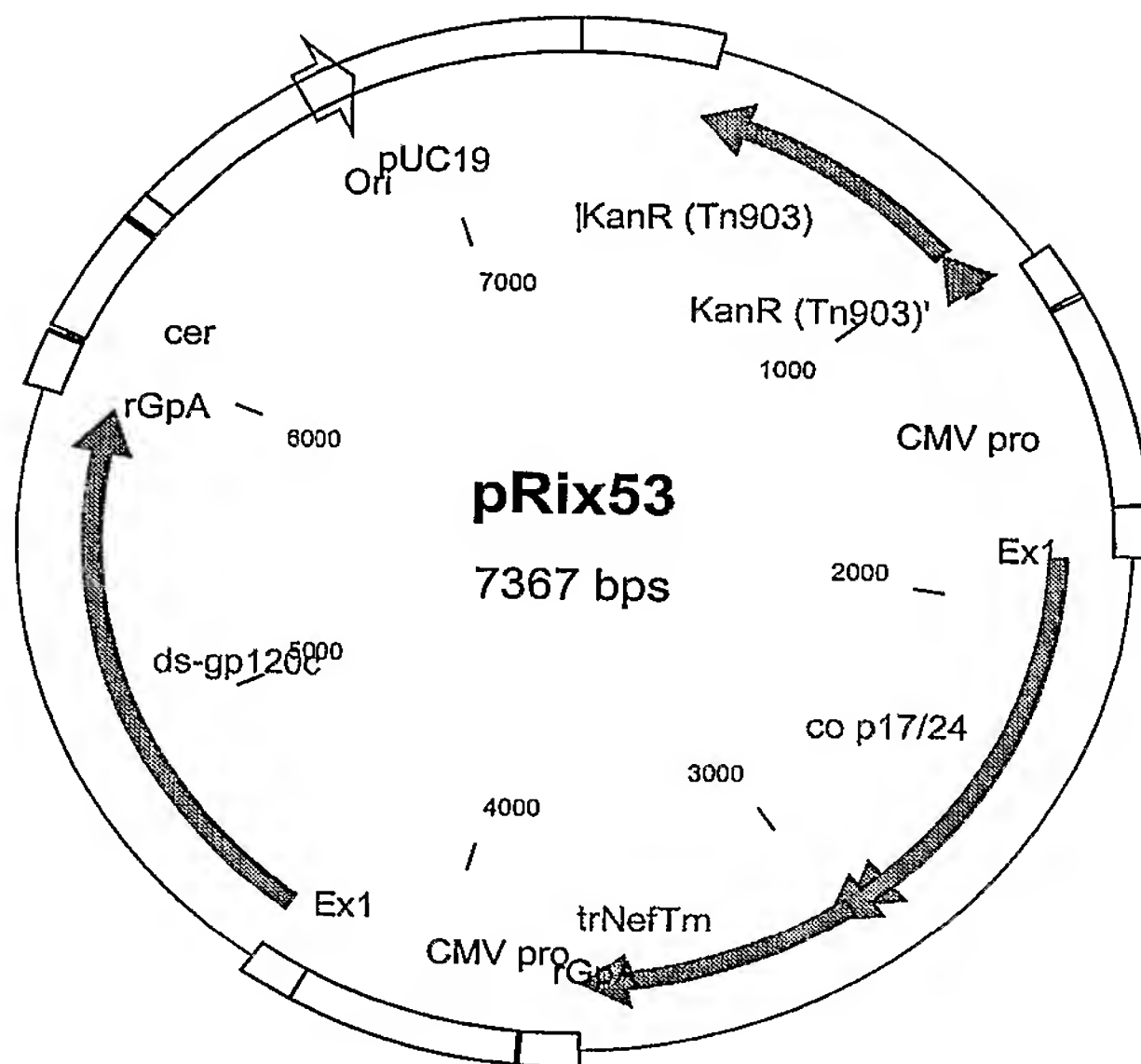


DNA and amino acid sequences of inserts:

Identical to p73I-GN2 and pRix12

Figure 25

pRix53

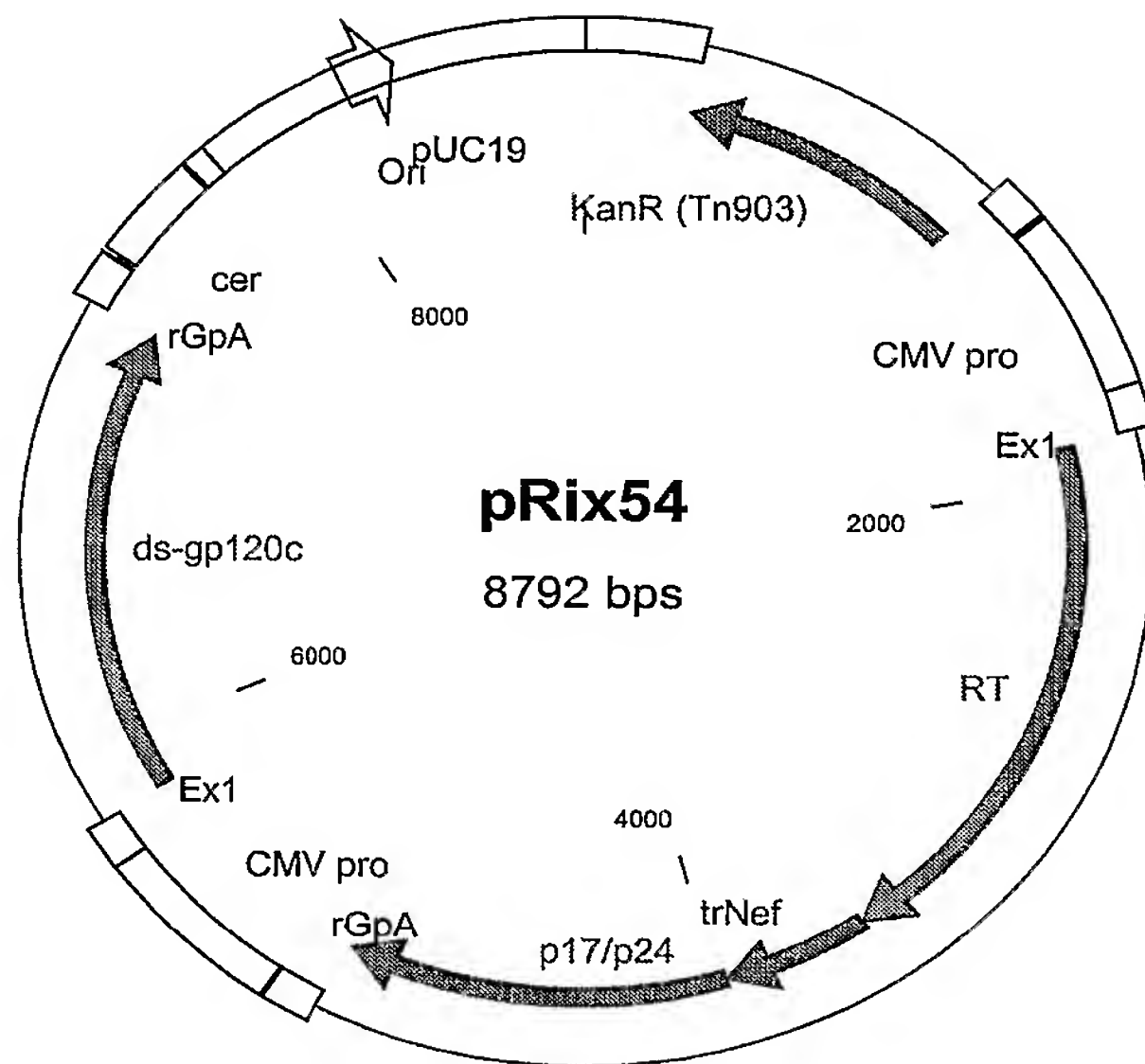


DNA and amino acid sequences of inserts:

Identical to pRix52 and pRix12

Figure 26

pRix54

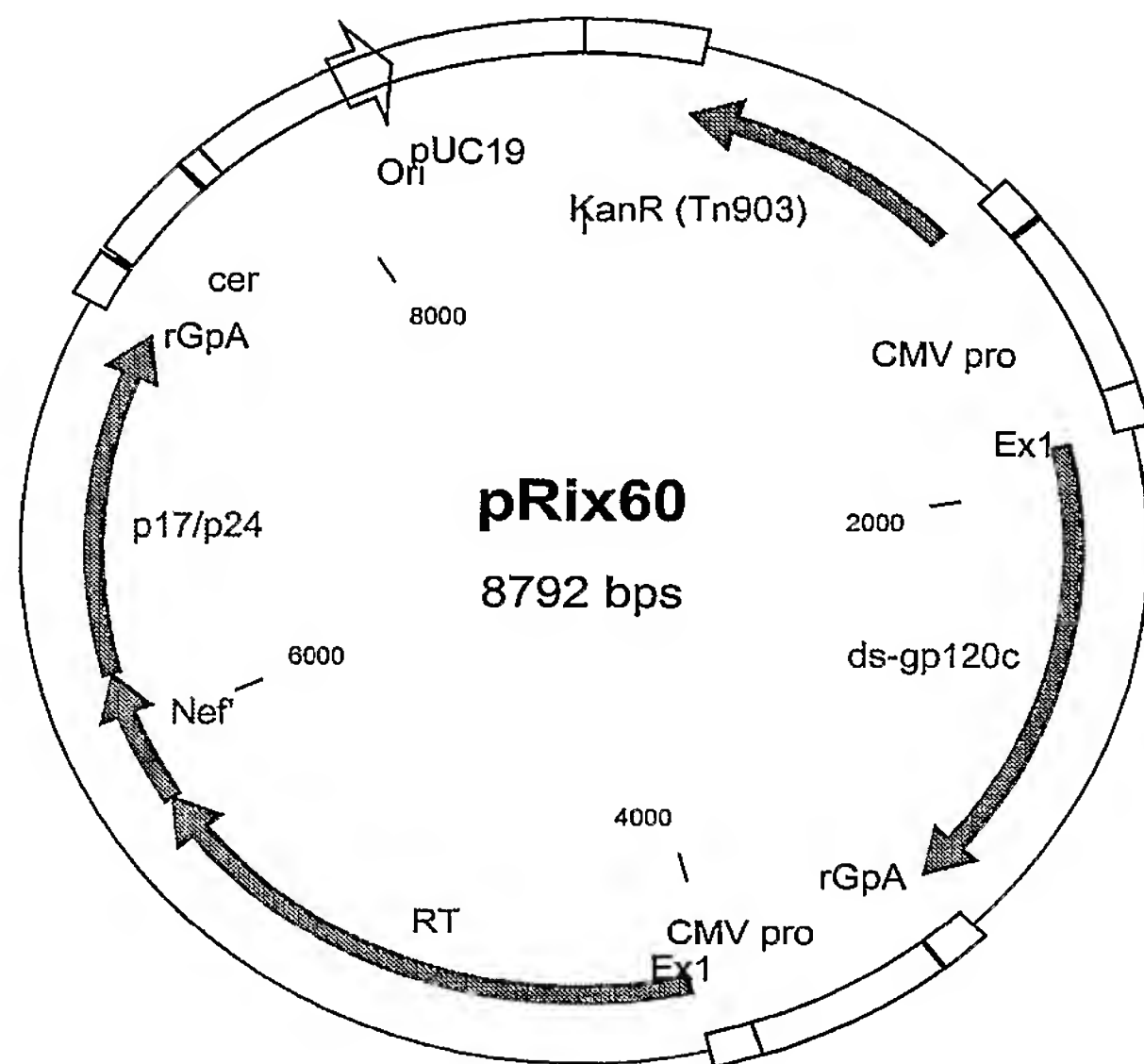


DNA and amino acid sequences of inserts:

Identical to pT-RNG and pRix12

Figure 27

pRix60

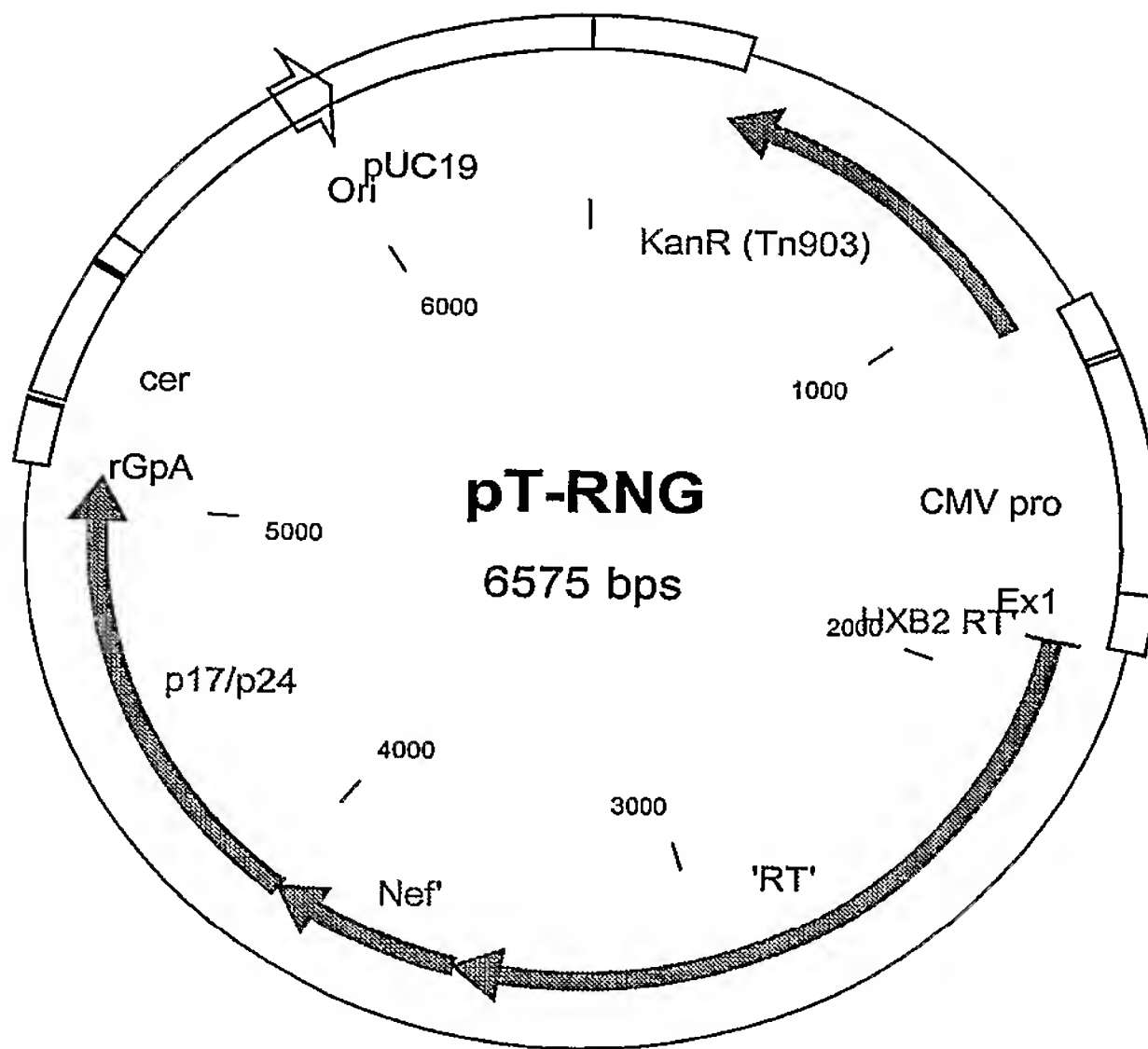


DNA and amino acid sequences of inserts:

Identical to pRix12 and pT-rng

Figure 28

pT-RNG



DNA sequence of insert:

```

ATGGGCCCCATCAGTCCCATCGAGACCGTGCCGGTGAAGCTGAAACCCGGGATGGACGGCCCCAAGGTCAA
GCAGTGGCCACTCACCGAGGAGAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAAGAGGGCA
AGATCAGCAAGATCGGGCCTGAGAACCATAACAACCCCCCGTGTGTTGCCATCAAGAAGAAGGACAGCACC
AAGTGGCGCAAGCTGGTGGATTTCGGGGAGCTGAATAAGCGGACCCAGGATTTCTGGGAGGTCCAGCTGGG
CATCCCCCATCCGGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGCTGGACGTGGGCGACGCTTACTTCA
GCGTCCCTCTGGACGAGGACTTTAGAAAGTACACCGCCTTTACCATCCCATCTATCAACAACGAGACCCCT
GGCATCAGATATCAGTACAACGTCCTCCCCCAGGGCTGGAAGGGCTCTCCCGCCATTTTCCAGAGCTCCAT
GACCAAGATCCTGGAGCCGTTTCGGAAGCAGAACCCCGATATCGTCATCTACCAGTACATGGACGACCTGT
ACGTGGGCTCTGACCTGGAAATCGGGCAGCATCGCACGAAGATTGAGGAGCTGAGGCAGCATCTGCTGAGA
TGGGGCCTGACCACTCCGGACAAGAAGCATCAGAAGGAGCCGCCATTCCTGAAGATGGGCTACGAGCTCCA
TCCCGACAAGTGGACCGTGCAGCCTATCGTCCTCCCCGAGAAGGACAGCTGGACCGTGAACGACATCCAGA
AGCTGGTGGGCAAGCTCAACTGGGCTAGCCAGATCTATCCCGGGATCAAGGTGCGCCAGCTCTGCAAGCTG
CTGCGCGGCACCAAGGCCCTGACCGAGGTGATTCCCCTCACGGAGGAAGCCGAGCTCGAGCTGGCTGAGAA
CCGGGAGATCCTGAAGGAGCCCGTGCACGGCGTGTACTATGACCCCTCCAAGGACCTGATCGCCGAAATCC
AGAAGCAGGGCCAGGGGCAGTGGACATAACAGATTTACCAGGAGCCTTTCAAGAACCTCAAGACCGGCAAG
TACGCCCCGATGAGGGGCGCCACACCAACGATGTCAAGCAGCTGACCGAGGCGGTCCAGAAGATCACGAC
CGAGTCCATCGTGATCTGGGGGAAGACACCCAAGTTCAAGCTGCCTATCCAGAAGGAGACCTGGGAGACGT
GGTGGACCGAATATTGGCAGGCCACCTGGATTCCCGAGTGGGAGTTTCGTGAATACACCTCCTCTGGTGAAG
CTGTGGTACCAGCTCGAGAAGGAGCCCATCGTGGGCGCGGAGACATTCTACGTGGACGGCGCGGCCAACC
CGAAACAAAGCTCGGGGAAGGCCGGGTACGTACCAACCGGGGCGCCAGAAGGTCGTCACCCTGACCGACA
CCACCAACCAGAAGACGGAGCTGCAGGCCATCTATCTCGCTCTCCAGGACTCCGGCCTGGAGGTGAACATC
GTGACGGACAGCCAGTACGCGCTGGGCATTATTACAGGCCAGCCGGACAGTCCGAGAGCGAACTGGTGAA
CCAGATTATCGAGCAGCTGATCAAGAAAGAGAAGGTCTACCTCGCCTGGGTCCCGGCCCATTAAGGGCATTG
GCGGCAACGAGCAGGTGCACAAGCTGGTGAAGTGCGGGGATTAGAAAGGTGCTGATGGTGGGTTTTCCAGTC
ACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTAAAGAAAA
GGGGGGACTGGAAGGGCTAATTCCTCCCAAAGAAGACAAGATATCCTTGATCTGTGGATCTACCACACAC
AAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACCTTTGGATGG
TGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGT

```

Figure 28 continued

ACACCCTGTGAGCCTGCATGGGATGGATGACCCGGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCC
 TAGCATTTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCATGGGTGCCCCGAGCTTTCG
 GTACTGTCTGGTGGAGAGCTGGACAGATGGGAGAAAATTAGGCTGCGCCCCGGGAGGCAAAAAGAAATACAA
 GCTCAAGCATATCGTGTGGGCCTCGAGGGAGCTTGAACGGTTTGCCGTGAACCCAGGCCTGCTGGAAACAT
 CTGAGGGATGTCGCCAGATCCTGGGGCAATTGCAGCCATCCCTCCAGACCGGGAGTGAAGAGCTGAGGTCC
 TTGTATAACACAGTGGCTACCTCTACTGCGTACACCAGAGGATCGAGATTAAGGATACCAAGGAGGCCTT
 GGACAAAATTGAGGAGGAGCAAAACAAGAGCAAGAAGAAGGCCAGCAGGCAGCTGCTGACACTGGGCATA
 GCAACCAGGTATCACAGAACTATCCTATTGTCCAAAACATTCAGGGCCAGATGGTTCATCAGGCCATCAGC
 CCCCAGGACGCTCAATGCCTGGGTGAAGGTTGTGAAGAGAAGGCCTTTTCTCCTGAGGTTATCCCCATGTT
 CTCCGCTTTTGAGTGAGGGGGCCACTCCTCAGGACCTCAATACAATGCTTAATACCGTGGGCGGCCATCAGG
 CCGCCATGCAAAATGTTGAAGGAGACTATCAACGAGGAGGCAGCCGAGTGGGACAGAGTGCATCCCGTCCAC
 GCTGGCCCAATCGCGCCCGGACAGATGCGGGAGCCTCGCGGCTCTGACATTGCCGGCACCACCTCTACACT
 GCAAGAGCAAATCGGATGGATGACCAACAATCCTCCCATCCCAGTTGGAGAAATCTATAAACGGTGGATCA
 TCCTGGGCCTGAACAAGATCGTGCAGCATGTACTCTCCGACATCCATCCTTGACATTAGACAGGGACCCAAA
 GAGCCTTTTAGGGATTACGTGACCGGTTTTATAAGACCCTGCGAGCAGAGCAGGCCTCTCAGGAGGTCAA
 AAATGGATGACGGAGACACTCCTGGTACAGAACGCTAACCCCGACTGCAAAACAATCTTGAAGGCACTAG
 GCCCGGCTGCCACCCTGGAAGAGATGATGACCGCCTGTCAGGGAGTAGGCGGACCCGGACACAAAGCCAGA
 GTGTTGTAA [SEQ ID NO: 88]

Amino acid sequence of insert:

MGPISPIETVPVKLKPGMDGPKVKQWPLTEEKIKALVEICTEMEKEGKISKIGPENPYNTPVFIAIKKKDST
 KWRKLVDLFRELNKRQDFWEVQLGIPHPAGLKKKKSVTVLVDVGDAYFSVPLDEDFRKYTAFTIPSINNETH
 GIRYQYNVLPQGWKGSPAIFQSSMTKILEPFRKQNPDIVIYQYMDDLIVGSDLEIGQHRTKIEELRQHLLR
 WGLTTPDKKHQKEPPFLKMGYELHPDKWTVQPIVLPEKDSWTVNDIQKLVGKLNWASQIYPGIKVRQLCKL
 LRGTKALTEVIPLTEEALELELAENREILKEPVHGVYDPSKDLIAEIQKQGQGWYQIYQEPFKNLKTGK
 YARMGAHTNDVKQLTEAVQKITTESIVIWGKTPKFKLPIQKETWETWWTEYWQATWIPEWEFVNTPPLVK
 LWYQLEKEPIVGAETFYVDGAANRETKLGKAGYVTNRGRQKVVTLTDTTNQKTELQAIYLALQDSGLEVNI
 VTDSQYALGIIQAQPDQSESELVNQIIIEQLIKKEKVYLAWVPAHKGIGGNEQVDKLVSAGIRKVL MVGFPV
 TPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLDWIYHTQGYFPDWQNYTPGPGVRYPLTFGW
 CYKLVPVEPDKVEEANKGENTSLHPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCMGARAS
 VLSGGELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLETSEGCRQILGQLQPSLQTGSEELRS
 LYNTVATLYCVHQRIEIKDTKEALDKIEEEQNKSKKKAQQAADTGHSNQVSQNYPIVQNIQGQMVHQAI
 PRTLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNMTLNTVGGHQAAQMLKETINEEAAEWDRVHPVH
 AGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGLNKIVRMYSPTSILDIRQGP
 EPFRDYVDRFYKTLRAEQASQEVKNWMTETLLVQNANPDCKTILKALGPAATLEEMMTACQGVGGPGHKAR
 VL [SEQ ID NO: 89]

Figure 29

A schematic representation of the constructs and associated expression data is shown below

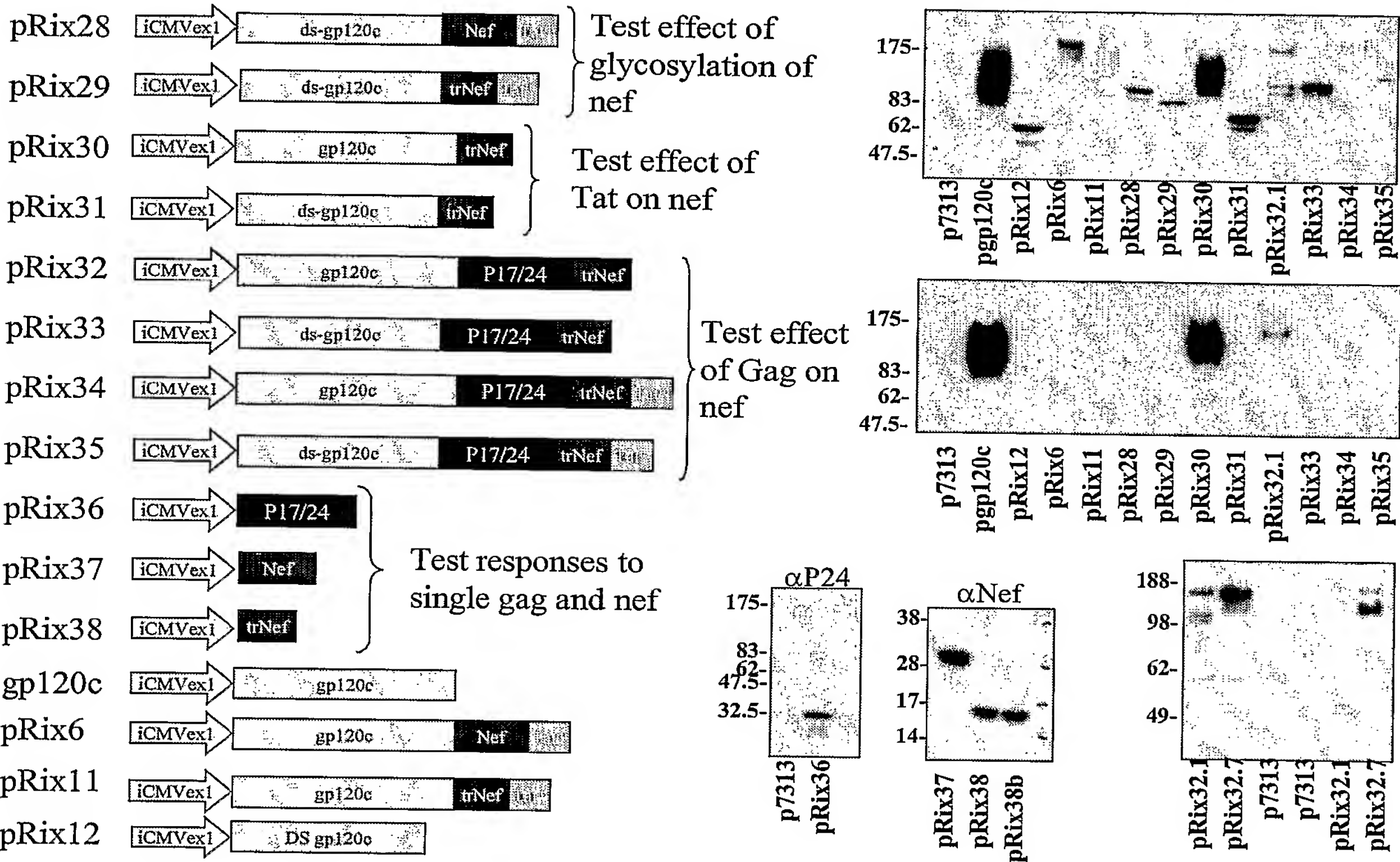
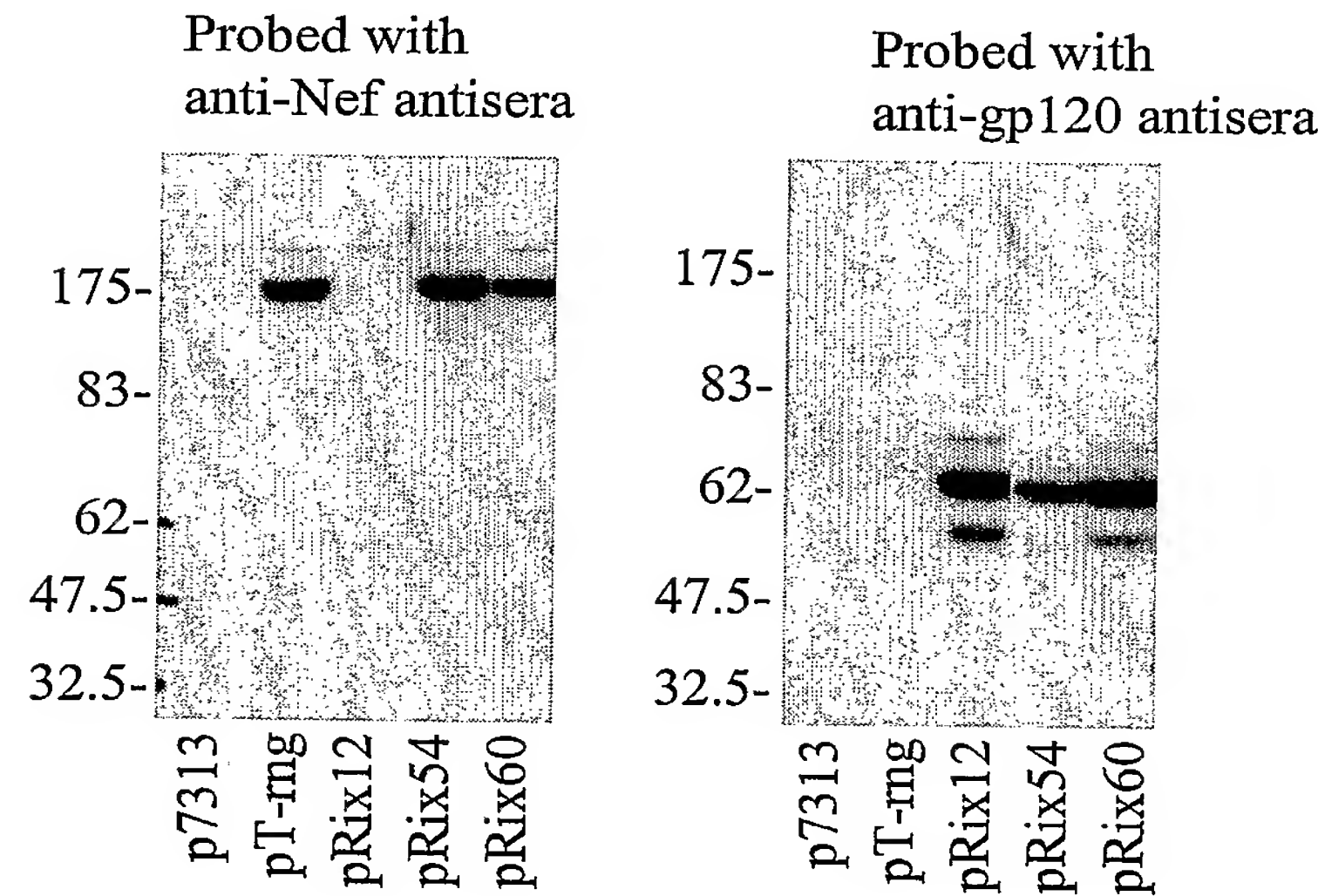


Figure 30

A schematic representation of further constructs and associated expression data:



Western blots probed with anti-nef (left) or anti-gp120 (right) antisera showing the expression of RNG and ds-gp120 from dual promoter and single vectors.

Plasmid schematics:

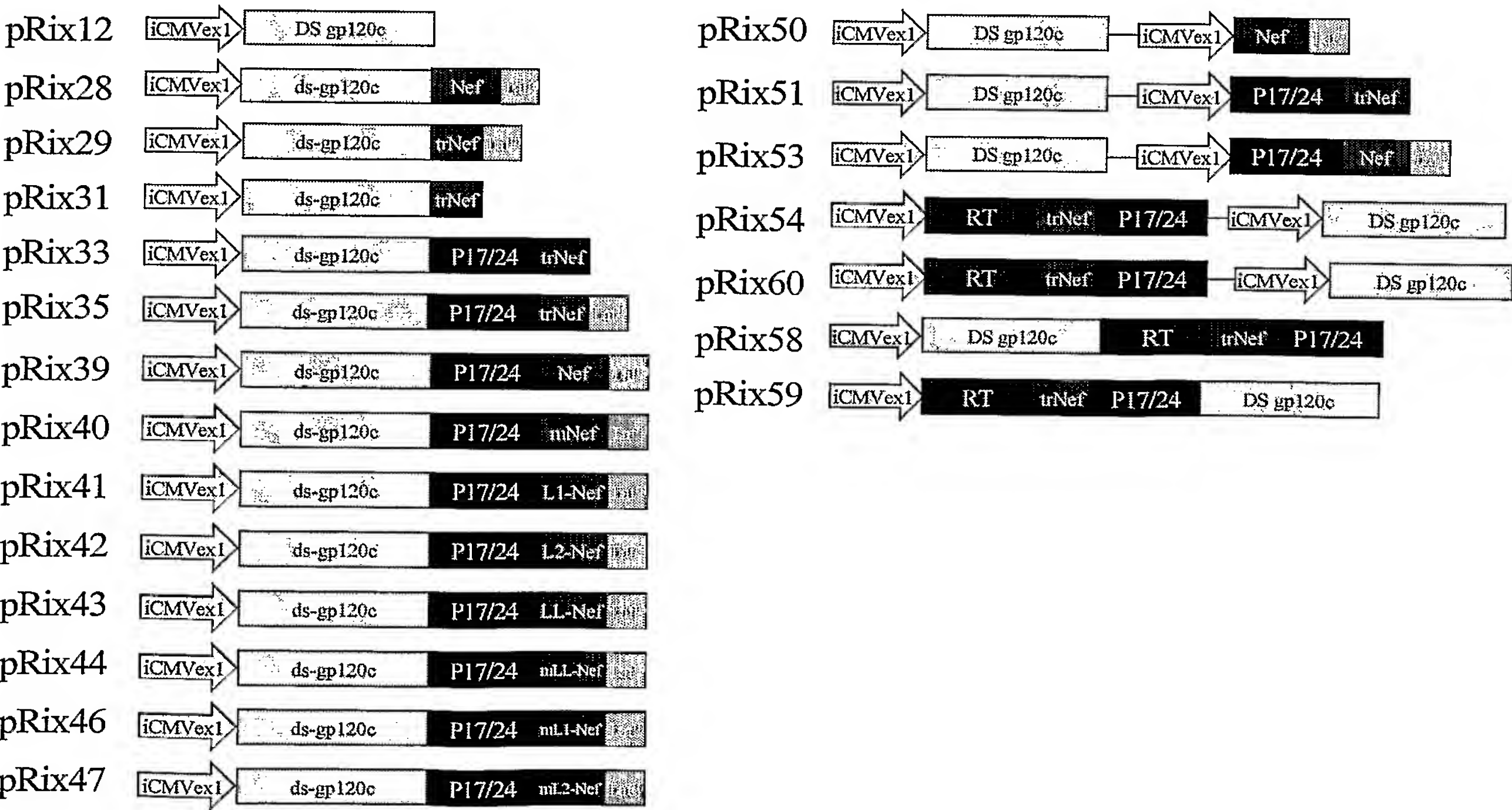
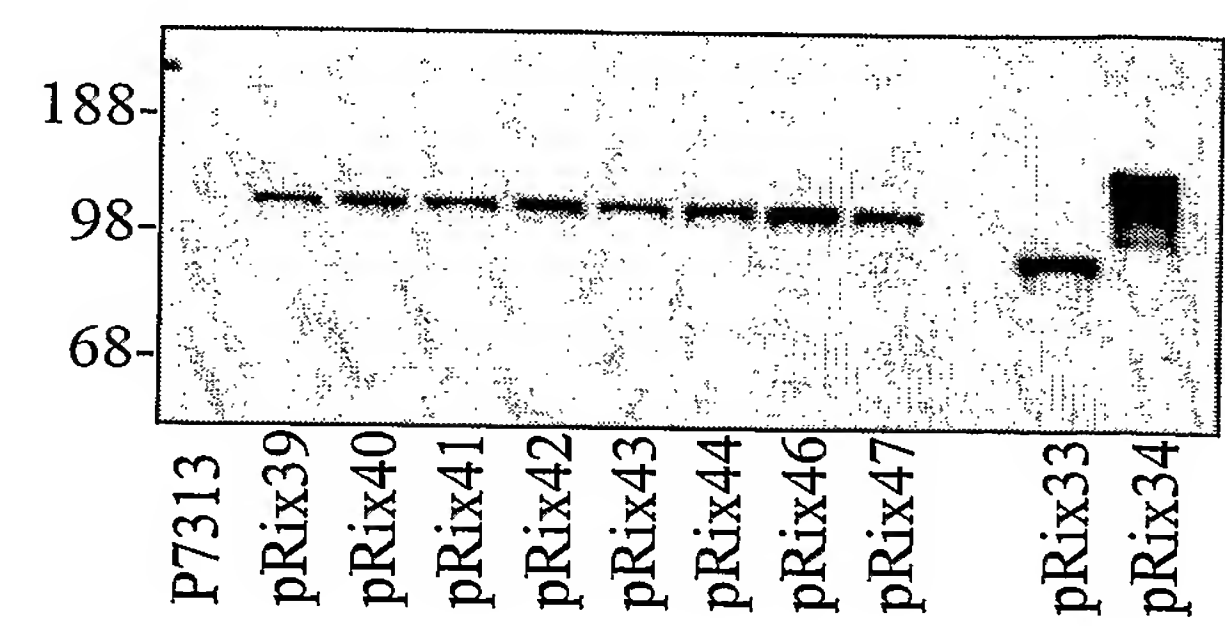


Figure 31

Expression data (anti-Nef) for dsGP120/Gag/Nef/Tat fusions with mutations in Nef (pRix 40-47)



Expression data (anti-Nef and anti-gp120) for dual promoter vectors

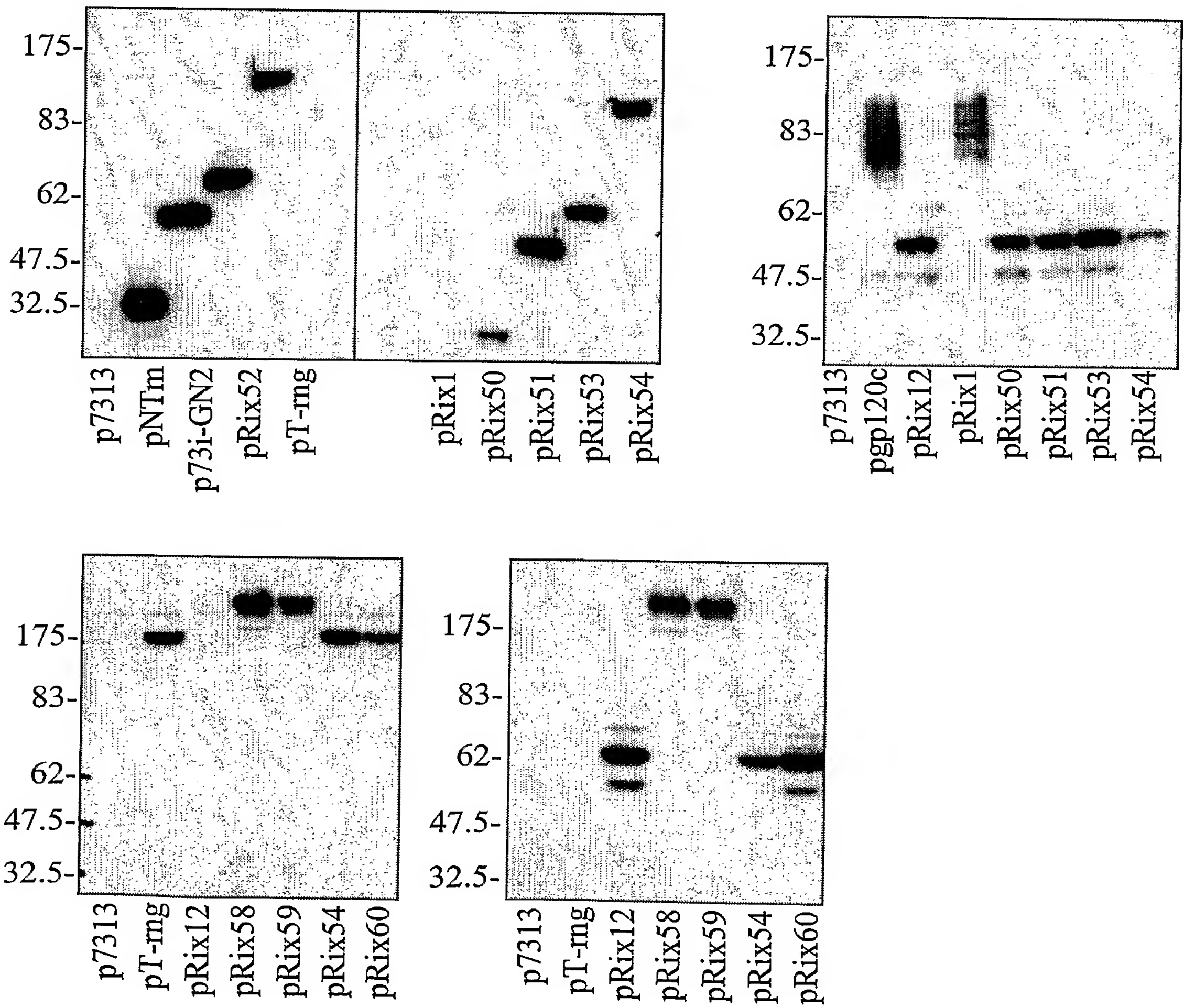


Figure 32

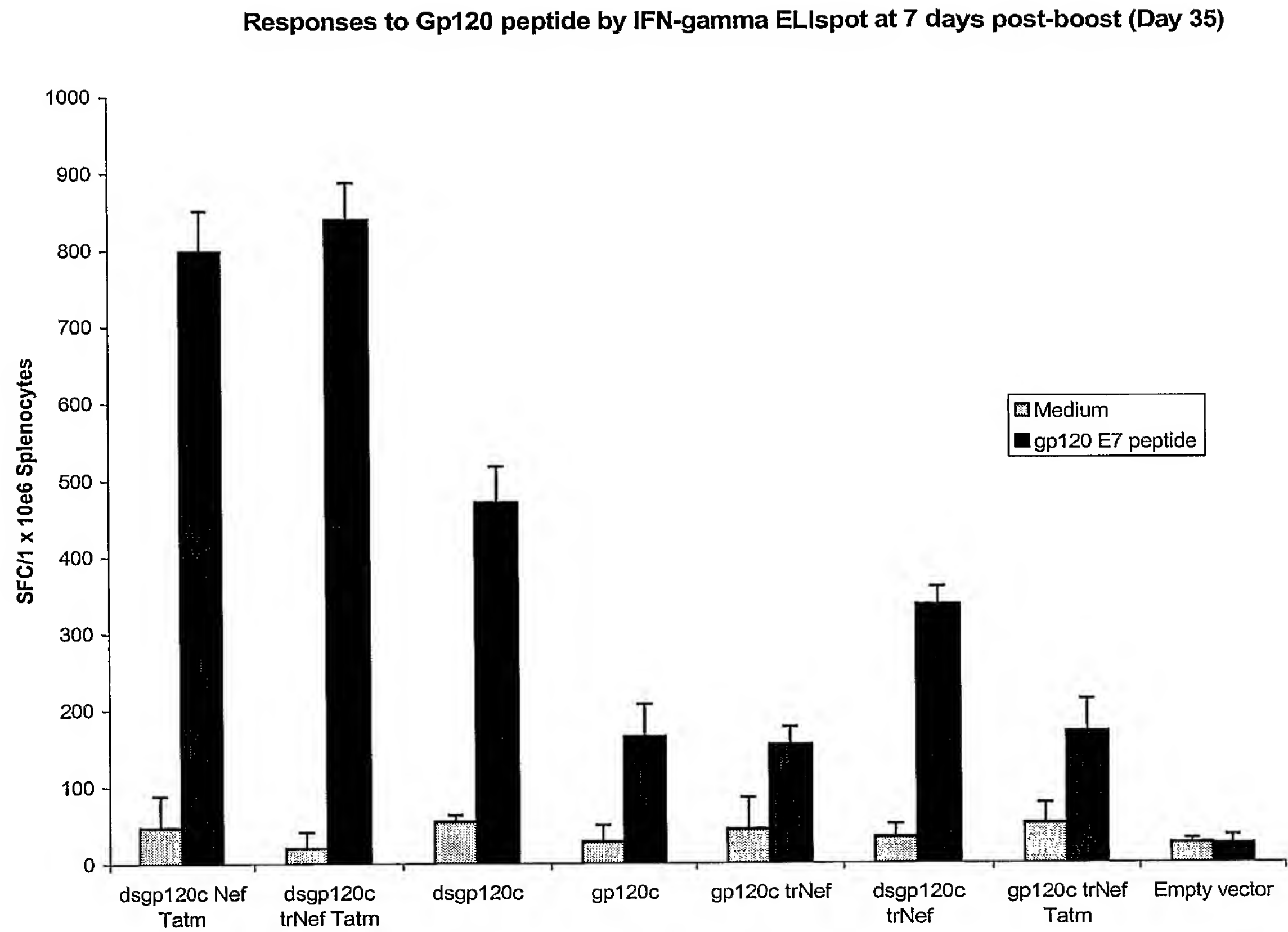


Figure 33

Responses to gp120 peptide by IFN-gamma ELIspot at 7 days post-boost (Day 35)

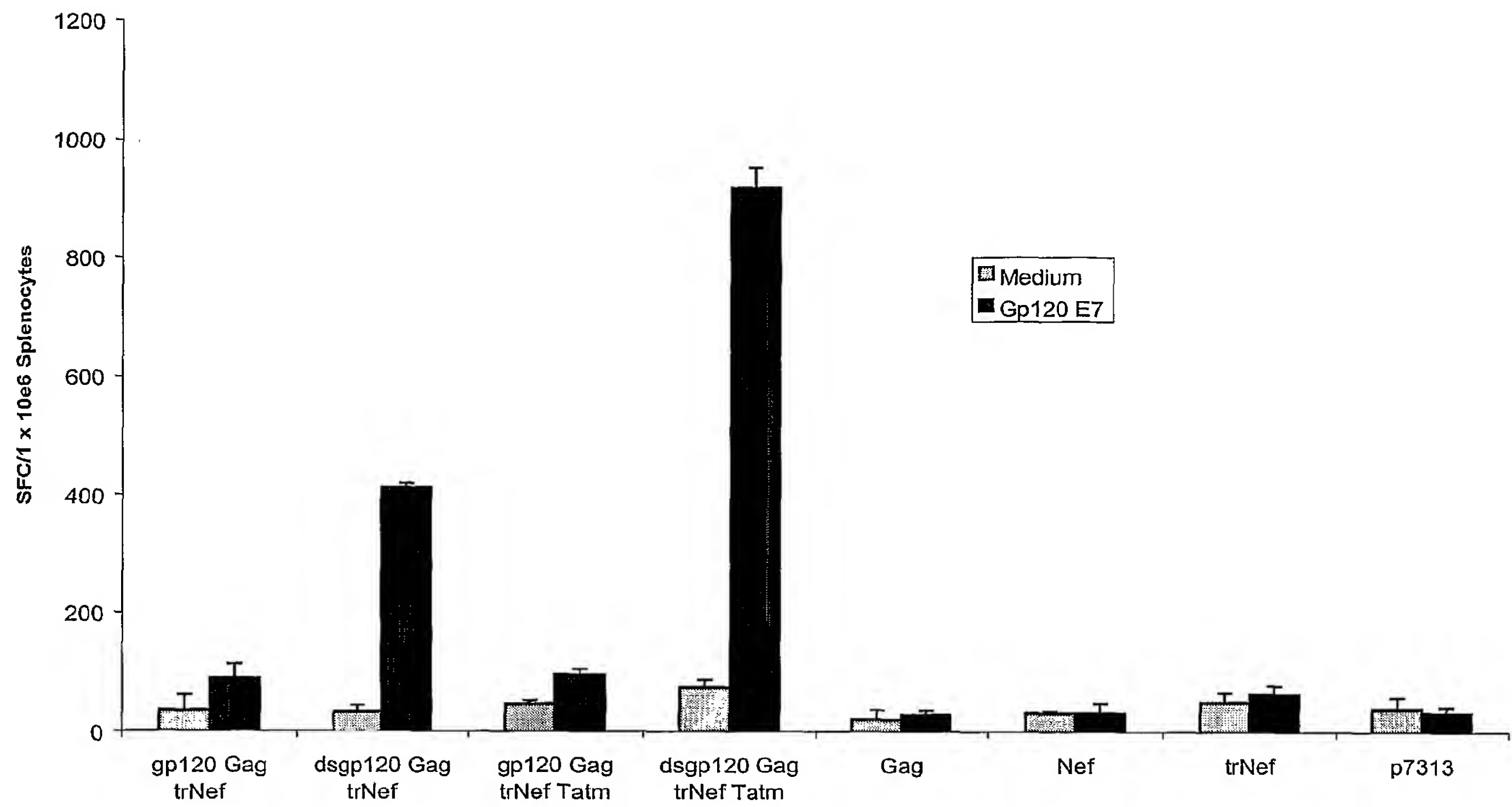


Figure 34

Response to in vitro restimulation with Gp120, Gag and RT peptides at 7 days post-boost (Day 35)
using IFN-gamma ELISpot

